# ANALYSIS OF A TEMPERATURE SENSITIVE MUTATION AFFECTING ALDEHYDE OXIDASE ACTIVITY IN *DROSOPHILA MELANOGASTER* ©

by

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ABSTRACT

A strain of Drosophila melanogaster (mid america stock culture no. h116) has been reported to be deficient in aldehyde oxidase activity (Hickey and Singh 1982). This strain was characterized during the course of this study and compared to other mutant strains known to be deficient in aldehyde oxidase activity. During the course of this investigation, the h116 strain was found to be temperature sensitive in its viability. It was found that the two phenotypes, the enzyme deficiency, and the temperature sensitive lethality were the result of two different mutations, both mapping to the X-chromosome. These two mutations were found to be separable by recombination. The enzyme deficiency was found to map to the same locus as the cinnamon mutation, another mutation which affects aldehyde oxidase production. The developmental profile of aldehyde oxidase in the h116 strain was compared to the developmental profile in the Canton S wild type strain. The aldehyde oxidase activity in adult h116 individuals was also compared to that of various other strains. It was also found that the aldehyde oxidase activity was temperature sensitive in the adult flies.

The temperature sensitive lethality mutation was mapped to position 1-0.1.

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# INTRODUCTION

One of the most intensively studied areas in biology today is that of the mechanism of gene regulation. Throughout the earlier and middle parts of this century, genetic research had focused on defining exactly the nature of the gene, with regards to its chemical composition, structure, and its transmission in populations. Now research has focused on exactly how genes function, and how they are regulated. Particularly within the last few years, with the development of powerful tools, such as recombinant DNA techniques, DNA sequencing, and restriction enzyme mapping, much has been learned about the molecular basis of gene action. However, this research has not yet yielded an answer to the basic question of how eukaryotic genes are regulated.

Over the last few years much has been learned about the regulation of prokaryotic genes. This is due to their comparatively simple chromosome structure, which is basically a naked strand of DNA, and their few number of genes. The eukaryotic genome, on the other hand is much more complex. This is due to several factors. The first is the structure of the chromatin itself. Histones and other proteins are bound to the DNA, and together form nucleosomes. The arrangement of the genes can be very different, with the

genes being sometimes arranged in tandem repeats. The sequence of the gene itself is much more complex, often being interrupted by intervening non-translated sequences.

Although there is a large difference between the prokaryotes and eukaryotes as far as chromosome structure and gene arrangement is concerned, the method of studying the regulation of genes is still the same. This method is to choose an enzyme or a protein, and study factors which affect its production or activity (Doane 1969). In particular, the identification of mutants which affect the activity of the gene has proved useful. Once the mutants are recovered, they can be compared to each other, and to the normal system in order to infer how the gene is regulated. In this manner, Jacob and Monod (1961) were able to determine how the lac operon functioned in E.Col: At present the same techniques are employed to discover how eukaryotic gene systems work. These eukaryotic systems will be dealt with in more detail in the next section.

One of the most extensively studied gene regulatory systems in eukaryotes is that of the aldehyde oxidase, xanthine dehydrogenase, pyridoxal oxidase system in Drosophila melanogaster. This project is concerned with the characterization of a regulatory mutation in a strain of

Drosophila melanogaster, which affects the activity of aldehyde oxidase and xanthine dehydrogenase. The mutant will be compared with other mutations in the system and the nature of its possible mode of action will be discussed.

# LITERATURE REVIEW

## 1. Introduction

One of the central problems in molecular biology today is determining how genes are regulated. Since the first operons were discovered in the early 1960s much more has been learned about the nature of gene regulation both in prokaryotes and eukaryotes. The solutions to the question of gene regulation were first found in the prokaryotes. However, the solutions to the question in eukaryotes has proved to be much more difficult to answer. In the following literature review, a brief examination of the research and theory of gene regulation will be presented.

## 2. Prokaryotic Gene Regulation

The first answers to the question of gene regulation were found in the prokaryotes. Jacob and Monod (1961) proposed a model of gene regulation by which prokaryotic structural genes could be regulated. This was termed the operon model. The operon is defined as a group of genes, which are co-ordinately controlled by a single regulator (Watson 1975). The basis of this method of regulation was the existence of a regulatory protein, which acted to physically block transcription by blocking RNA polymerase from transcribing the structural genes (Rezinkoff, 1972).

In addition to the repressor molecule, there is also an effector molecule, which affects the affinity of the repressor for the operator region (Reznikoff, 1972). This effector molecule can be an inducer or a corepressor. The inducer acts to decrease the repressor's binding affinity, while the corepressor acts to increase the repressor's binding affinity.

One of the best known examples of this operon system is the lac operon in the bacterium E. coli (Jacob and Monod, 1961). In this system, the structural gene for the regulatory protein is located close to the structural genes which are involved in the utilization of lactose. These genes code for the enzymes *b-galactosidase, *b-galactoside permease, and thiogalactoside transacetylase. These three genes are commonly known as Z, Y, and A.

The RNA polymerase first binds to a promotor site next to the gene for the regulatory protein. As the RNA polymerase moves along the DNA strand, the gene coding for the repressor is encountered, transcribed and translated into the repressor protein. This then acts as the regulatory protein, which controls transcription. After the repressor protein is transcribed, there is a terminator sequence, which stops transcription. Immediately adjacent to this sequence is the promotor site for the Z, Y and A

genes. Located between the promotor site, and the structural genes, is a site known as the operator site. In the absence of lactose, the regulatory protein binds to the operator site. When the RNA polymerase binds to the promotor, it is physically blocked from transcription by the repressor. In the presence of lactose, the repressor will preferentially bind to the lactose, which renders it unable to bind to the operator. Therefore, the RNA polymerase is free to transcribe the three genes. In this instance, the lactose molecule acts as the effector molecule, which alters the affinity of the repressor for the operator site (Rezinkoff 1972)

Since the demonstration of the lac operon, many more operons have been described in bacteria, such as the tryptophan operon (Rose et al., 1973), and the arabinose operon (Greenblatt and Schlief, 1971). Operons have also been found in viruses (Maniatis et al., 1975).

In eukaryotic systems, the solution to gene regulation is much more complex. This is primarily due to the increased complexity of the genome itself. In the next section, a brief review of eukaryotic gene regulation will be given.

## 3. Eukaryotic Gene Regulation

The study of eukaryotic gene regulation has shown that, although there are parallels, eukaryotic genes are not regulated in the same manner as the simple operons of the prokaryotes. Once the primary transcript of a structural gene is made, it often undergoes more modification. This is what Paigen (1979) refers to as the "realization of enzyme activity".

### 3a. Levels of Gene Control

One of the main reasons for the differences in the regulation of genes in prokaryotes and eukaryotes, is the increased complexity of the eukaryotic genome (Darnell 1982). This increase in complexity also increases the levels at chich the genes may be controlled. Whereas prokaryotic gene regulation occurs only at transcription, eukaryotic gene regulation can occur at many different levels (Darnell 1982)

The first level of control in the eukaryotes, is the choice of the RNA polymerase (Darnell 1982). Unlike prokaryotes, eukaryotes have three different types of polymerases. Polymerase I, and polymerase III, are employed in the transcription of ribosomal RNA, and transfer RNA, respectively,

while polymerase II is responsible for the transcription of genes which code for proteins (Roeder 1976, Corden et al. 1980). The RNA polymerase II recognizes an initiation site 8 to 10 nucleotides in length, in which the nucleotides TATA are strongly conserved (Corden et al 1980). It is widely thought that this step, the initiation of transcription, is the major controlling step in transcriptional control (Darnell 1982). However, Darnell (1982) also points out that termination of transcription, is another potential step in transcriptional control.

The next level of control is the processing of the primary RNA transcript into mRNA. Even before the primary RNA transcript has reached a length of 50 nucleotides, a m7Gp7 cap is added on to the first nucleotide of the RNA chain (Darnell 1982). After the gene has been transcribed, a poly A cap is added on to the primary RNA transcript (Brawerman 1981). This then is the heterogeneous nuclear RNA transcript. The addition of the two caps is a rapid event (Darnell 1982). The next step in the processing of the RNA is the splicing of the non coding regions out of the transcript, to produce the mRNA (Darnell 1982). After the mRNA has been produced, the next level of control is translational control, where the protein is first produced. However, this is not the final level of control of gene expression. Once a gene has produced a protein, it can still

be subject to further processing (Paigen 1979). In the next section, the genes which control regulation will be discussed.

3b. Regulatory Genes

In eukaryotes, there are many different classes of genes which potentially can be involved in the production of a protein. The first class is the structural gene, which is the DNA sequence coding for the primary translation product itself (Paigen, 1979). Courtright (1975), outlines the various indirect criteria by which the structural gene can be determined. These are: (a) alteration in the kinetic properties of the enzymes in mutants, (b) lateration in mutants of the electrophoretic mobility, (c) proportionality of enzyme activity with gene dosage, (d) codominant expression of allelic cistrons in heterozygotes, ie heterozygotes should show intermediate enzyme activity to that of the two homozygotes, (e) hybrid enzyme formation in cases where the enzyme is a dimer or multimer. The satisfaction of one of these criteria is an indication that the gene is in fact a structural gene.

The next class of genes are the processing genes. These are the genes which are involved in what Paigen (1979) terms the realization of enzyme activity. These are grouped

in several categories. The first category is the modifier genes. These are the genes which act to covalently modify the primary amino acid sequence, either by removing amino acids from the sequence, or adding various groups, such as sugars, to the sequence. The next category of processing genes are the architectural genes. These genes are involved in forming the three dimensional structure of the protein, and the intracellular localization of the protein.

The third class is known as the regulatory genes, which are involved in the regulation of protein synthesis. There are three types of regulatory genes. The first type is the systemic regulators; these are the genes which affect the synthesis of the primary sequence. There are several stages where the genes can potentially be regulated by a systemic regulator, as has been discussed above.

The second category of regulatory genes is the inducibility genes, which affect the initiation of synthesis of the structural gene. The third category is the receptor genes which are involved in the coding of receptor proteins and the responses to effector molecules. The fourth class of regulator genes are the temporal genes, which affect the developmental regulation.

An example of these regulatory and structural genes is seen in Paigen's work on the acid hydrolase *b-glucuronidase in mice. The structural gene was determined to be on the distal end of chromosome 5 (Paigen, 1961a). This was termed the gus locus. Shortly after a mutant was found to be involved in the developmental regulation of the enzyme, which mapped next to the structural gene (Paigen, 1961b). This was termed the gus-t locus. This mutant was characterized by a lower enzyme level in development. The next mutant found in this system was the Eg gene, which affected the anchoring of the enzyme in membranes (Glanschow and Paigen, 1967). The fourth gene in the system was found by Swank (1973), which affected the inducibility of the *b-glucuronidase by androgens. The last gene found in the system was the gur-u gene, which controls the systemic rates of enzyme synthesis (Paigen, 1979). Therefore, to sum up, in the *b-glucuronidase system, there is the structural gene (gus), a developmental gene (gus-t), an architectural gene (Eg), an inducability gene (gus-r), and a systemic regulator gene (gur-u).

In the next section, the system of genes involved in the regulation of the molybdenum hydroxylases; aldehyde oxidase, xanthine dehydrogenase, and pyridoxal oxidase, in Drosophila, will be examined.

## 4. The Xanthine Dehydrogenase, Aldehyde Oxidase, and Pyridoxal Oxidase System

### 4a. History of the System

The first mutant in this system was discovered in 1956 by Forrest et al. It was found that a strain of Drosophila which had brownish eyes, was also lacking Xanthine dehydrogenase activity. The brownish eye colour was due to a lack of the red pigment drosopterine. The absence of the xanthine dehydrogenase was found to be the reason for the lack of the pigment. The specific reaction which was blocked was the conversion of 2-amino 4-hydroxy xanthopteridine to isoxanthopteridine. The mutation responsible for the lack of the xanthine dehydrogenase was mapped to position 3-52.0, and was named the rosy locus. Later analysis (Glassman, 1961) showed this to be the structural gene for the enzyme.

A second mutant in this system was described by Glassman and Mitchell (1959). This mutant was known as maroon-like. It had been known since 1935 (Linsey and Grell, 1968) as a mutation which caused homozygous adults to have maroon coloured eyes. This mutation mimicked a different third chromosome mutation known as maroon which

caused the adult flies to have maroon eyes also. These maroon-like flies were found to also be deficient in xanthine dehydrogenase activity (Glassman and Mitchell, 1959). This mutant was found to be morphologically indistinguishable from the rosy mutation, but was found to map to the X-chromosome, at position 64.8 (Glassman, 1962) An analysis of immunologically cross reacting material (CRM) in rosy and maroon-like mutants showed that CRM was present in maroonlike flies, but absent in the rosy flies. This indicated that the maroon-like mutation was involved in the realization of the enzyme activity, and not the structural locus.

The maroon-like locus was also found to be involved in the production of pyridoxal oxidase, as well as xanthine dehydrogenase (Forrest et al.,1961). A third enzyme, aldehyde oxidase was also found to be controlled by the maroon-like locus (Courtright, 1967). The structural genes for both these enzymes were subsequently mapped to position 3-57, and were termed, lpo for pyridoxal oxidase, and aldox for aldehyde oxidase (Collins and Glassman, 1969; Dickinson, 1970).

A second regulatory mutant was found by Keller and Glassman (1964), which was found to regulate the activity of all three enzymes. However, it did not completely

eliminate all three enzymes, but reduced their activity greatly. This site was named low xanthine dehydrogenase (lxd), and was found to map to position 3-33. A fourth regulatory site was reported by Baker (1973) termed cinnamon due to the eye colour in homozygous adult flies. This locus mapped to the X-chromosome at position 0.0. This mutant was also characterized by the lack of activity for all three enzymes. The most recent mutant found in this system was the aldox-2 mutant, reported by Bently and Williamson (1979). This mutant was mapped to position 2-86, and was characterized by affecting aldehyde oxidase activity, and not xanthine dehydrogenase activity.

Therefore in summary, the xanthine dehydrogenase, aldehyde oxidase and pyridoxal oxidase enzyme system involves three structural genes, termed rosy, aldox and Ipo, and a total of four regulatory sites, maroon-like (ma-l), cinnamon (cin), low xanthine dehydrogenase (lxd) and aldox-2. These genes, and how they interact will be reviewed in more detail in the next subsections.

## 4b. The Structural Genes

The first described and best characterized locus in the whole system is the rosy locus at position 3-52. Further cytological evidence has shown it to be located

at the cytological position 87D8-12 (Lefevre, 1971). As stated previously, this locus was determined to be the structural locus for the xanthine dehydrogenase enzyme. This was determined on the basis of two criteria. The first was that electrophoretic variants of the enzyme mapped to this site (Chovnik et al., 1977). The second criterion was that gene dosages of 0 to 3 genes resulted in a corresponding change in the level of xanthine dehydrogenase activity (Gress, 1962). These two criteria fit Courtright's criteria for the termination of a structural gene (see section 2 of this literature review). The xanthine dehydrogenase enzyme itself was determined to have a molecular weight of 250,000 (Glassman et al., 1966) to 300,000 daltons (Andres, 1976). This enzyme was found to be dissociable into two subunits each 160,000 daltons (Andres, 1976). Using a recombination analysis of the region, Chovnick et al (1977) were able to show that the enzyme was a homodimer, and not a heterodimer (i.e. the enzyme was made up of two identical subunits, not two different subunits).

Chovnick et al. (1977) were also able to demonstrate that there was a cis acting regulatory element directly adjacent to the structural gene. This was accomplished using a recombination analysis of null mutants of xanthine dehydrogenase. This control element

was estimated to be approximately 3.0 kilobases from the structural element.

Aldox was determined to be the structural gene for aldehyde oxidase by the mapping of null alleles and electrophoretic variants (Dickinson, 1970). Much less is known about the structural gene of aldehyde oxidase as compared with xanthine dehydrogenase. There is evidence that two forms of aldehyde oxidase exist in the adult fly. An allele of aldox, called lao (low aldehyde oxidase) was found to map to the structural region (Meidinger and Williamson, 1975). This mutant had about 30% of the activity of wild type aldehyde oxidase activity, but had 60% of wild type immunologically cross reacting material (Meidinger and Williamson, 1978). Further study by Williamson et al. (1978) showed that the two forms were separable electrophoretically, and had different heat labilities. However this evidence is by no means conclusive, and the lao mutant could represent a partial deletion in the structural gene.

The structural gene for pyridoxal oxidase is lpo, located at position 3-57, the same locus as the aldox locus. This is the least known enzyme in the system. The only evidence for lpo being the structural locus is

the reduction of pyridoxal oxidase activity in lpo homozygotes, and the fact that heterozygotes for lpo and the wild type allele have activities intermediate between that of wild type flies and lpo homozygotes (Collins and Glassman, 1969). Since pyridoxal oxidase and aldehyde oxidase map to the same locus, it is possible that they are the same protein. O'Brien and MacIntyre (1978) summarize the evidence against this. First, the substrates for the two enzymes are different. Secondly, aldox null flies have wild type levels of pyridoxal oxidase, and lpo flies have wild type levels of aldehyde pyridoxal oxidase. Fourthly, the two enzymes can be separated chromatographically.

4c. The Regulatory Genes

Before the effects of these genes are discussed, it must be emphasized that these are not regulatory genes in the sense that they regulate the transcription of the structural gene. These genes most probably act to modify the primary protein product of the mRNA. According to Paigen's definition of regulatory genes, they are involved in the realization of enzyme activity. It is in this sense that they are regulatory genes.

The best known regulatory mutant in this system is the maroon-like (ma-l) mutation. It is characterized by having no active xanthine dehydrogenase, aldehyde oxidase, or peridoxal oxidase activity, although there is immunologically cross reacting material (CRM) present for XDH near wild type levels, and Ao CRM to a lesser degree (Dickinson, 1970). Glassman (1965) found that in vitro complementation could be achieved when homogenates of ma-l homozygotes were mixed with ry homozygotes. This in vitro complementation suggested that there was an active ma-l factor which modified the ry product into active enzyme. This complementation factor was purified and found to be a multimer with a molecular weight of 250,000 d. (Glassman et al., 1966). Although its function is still not clear, it has been suggested that the mode of action of this factor is the donation of a small cofactor from the multimer to the apoenzyme (Glassman et al., 1966). The ma-l mutation also shows a maternal effect, in that homozygous ma-l offspring from heterozygous mothers have maternally derived XDH activity, through the third instar larva stage (Williamson and Browder, 1975). However, it is not clear what the maternal substance is which is passed on to the eggs, but experiments of maternally affected progeny from ry mothers demonstrate that the active XDH enzyme is not the substance which is passed on (Glassman et al., 1964; O'Brien and MacIntyre, 1978). Aldehyde oxidase was found

not to play a part in the maternal effect. Finally, the ma-1 mutation, when complemented with another ma-1 allele produces an XDH enzyme which is less heat stable than the wild type enzyme, thus indicating that the ma-1 co-factor must be part of the active XDH molecule itself (O'Brien and MacIntyre, 1978).

The lxd allele was discovered by Keller and Glassman in 1964. It was found to be similar to the ma-1 mutation in that it affected the three enzymes, but it differed because it did not completely shut off the enzymatic activities, XDH being reduced to about 50% of its wild type activity, and AO being reduced to 10% of its activity. Only trace amounts of PO were detected (Keller and Glassman, 1964). In heterozygotes, full complementation is not seen for AO, or PO, but is seen for Xdh (O'Brien and MacIntyre, 1978). Courtright (1967) found that the Crm levels were close to wild type for XDH, but were reduced for AO. When extracts from homozygous lxd flies was mixed with homozygous ry extracts no complementation was seen (Glassman et al., 1964).

The most recently discovered mutation in the system was the aldox-2 mutant, reported by Bentley and Williamson (1979a). This was mapped to position 2-86, and was found to reduce the AO level to 25 to 30% of that of wild type.

It had no effect on XDH. The aldehyde oxidase produced was found to be more heat labile than the wild type enzyme. The comparison of the developmental profile of the wild type enzyme and the aldox-2 enzyme showed that there were equivalent amounts of enzyme produced in the two genotypes, until the pupal stage, when the aldox-2 enzyme levels decreased.

The cinnamon allele was first described by Baker in 1973. Morphologically, cin adult flies have identical eye colour to that of ma-1. However, the mutation was found to map to the opposite end of the X-chromosome, at position O.O. This mutation is further characterized by having no XDH, AO, or PO activity (Baker, 1973; Browder and Williamson, 1976). Further examination of the development of cin flies showed that there was a maternal effect on levels of XDH activity (Browder and Williamson, 1976). This maternal effect is much more pronounced in the cin mutation than in the ma-1 mutation, with the cin mutant showing a large increase in XDH activity during the larval stages. The XDH activity is still detectable for a short period after eclosion.

Another aspect of the cin mutation, is that it is a homozygous lethal mutation. However, Bentley and Williamson (1979) were able to induce 16 different alleles

of cinnamon, with varying degrees of viability, and varying amounts of aldehyde oxidase activity. These alleles fell into four complementation groups. Two of the cin strains generated, the cin 9 and cin 11 strains, were found to produce small amounts of aldehyde oxidase.

It is difficult to classify these mutations according to Paigen's classes of regulatory genes, since not a great deal is known about most of these mutants. The ma-1 mutation, which is by far the best characterized mutant, could be in the modifier category of processing genes, since the addition of the co-factor may be involved in the covalent modification of the protein, or the ma-1 gene could be an architectural processing gene if the addition of the co-factor served to alter the three dimentional shape of the enzyme. For the rest of the mutants, the classification is much more difficult. However, some classes of regulatory genes can be excluded. None of the mutants seem to be temporal mutants. These mutants can fit into any of the other classes. Therefore until more research is done, they will have to remain unclassified. It should be noted however, that some of these mutants could represent more than one type of regulatory gene, and therefore, naturally fall into more than one class of gene.

## 5. The hl16 Strain

Recently, a naturally occuring mutation was found by Hickey and Singh (1982) which affected the aldehyde oxidase and xanthine dehydrogenase activity in the strain. In addition to the decreased enzyme activity, the strain had a maroon eye colour. This eye colour was found to be due to a third chromosomal mutation called maroon(MA) which was not involved in this system (see section 3a). The gene controlling the enzyme deficiency was found to be X-linked. In addition, the viability of this strain was found to be temperature sensitive. In the following subsection, a brief discussion of the nature of temperature sensitive mutations will be given.

### 5a. Temperature Sensitive Mutations

In general, temperature has two effects on living organisms. The first is that of a rate effect on the organism's metabolism, and the second effect is on the tertiary and quaternary structure of biochemically important molecules (Hochachka and Somero, 1973). The specific mode of action of temperature on the structure of these molecules is to destroy the weak bonds which are responsible for the integrity of the molecule's structure. This is

the basis of action of a group of mutations known as temperature sensitive mutations. A temperature sensitive mutation can be defined as a mutation whose expression is conditional on the temperature at which the organism is currently growing. This group of mutations was first discovered in the prokaryotes (Suzuki, 1970), but has been found in insects (Suzuki, 1970) and mammalian cell cultures (Linquist 1976).

There are many different reported cases of temperature sensitive mutations in Drosophila. These mutations affect a wide range of phenotypes such as eye colour (Howels, 1979; Puckett and Petty, 1980), or hormone production (Garen et al., 1977), but often the effect of the temperature sensitive mutation is so severe that it is lethal.

Suzuki (1970) has presented a map of the X-chromosome showing the distribution of temperature sensitive mutations in Drosophila melanogaster, showing that there are over 250 X-linked temperature sensitive mutations. These occur in the same proportion as autosomal temperature sensitive mutations (Suzuki, 1970).

Suzuki (1970) has outlined a procedure by which the developmental timing of action a temperature sensitive lethal mutations. These have been termed "shift up" and

and "shift down" experiments. The shift up experiment involves sequentially shifting synchronously growing cultures of flies up to the restrictive or lethan temperature, and determining the stage at which shifting the developing culture has no effect on the survivorship of the flies. Temperature sensitive lethal mutations which act at a certain stage in development will cause the death of cultures which have been shifted up to the lethal temperature before this stage has been reached. Cultures which have gone through the sensitive period at the permissive temperature will not be affected by being shifted to the lethal temperature. Therefore these shift up experiments show when the end of the lethal period occurs. In a similar fashion, shift down experiments, allow the beginning of the lethal period to be determined. In this experiment, synchronously growing cultures of flies are sequentially shifted down from the lethal temperature to the permissive temperature. Cultures which are shifted before the lethal period begins will survive, but once the lethal period is reached at the restrictive temperature, shifting the culture down to the permissive temperature will not rescue it. Therefore, the decrease in survivorship of sequentially shifted cultures indicates the beginning of the lethal period. Once the beginning and end of the lethal period have been determined , it should be possible to grow a culture at the lethal temperature until just before the lethal period,

then shift the culture to the permissive period until that developmental period has passed, then return the culture to the lethal temperature where it can complete the life cycle. Conversely, growing the culture at the permissive temperature, and shifting it to the lethal temperature for the lethal period only, then returning it back to the permissive temperature, should show the same lethal effects as if the culture were left at the lethal temperature.

The purpose of this study therefore is to characterize the new mutation causing reduced levels of aldehyde oxidase and xanthine dehydrogenase (discovered by Hickey and Singh) and to compare it to the known regulatory mutations. As previously stated, with the exception of the ma-1 mutation, little is known about the action of the regulatory mutants. The h116 strain represents a new type of mutant, which may give further insight into the workings of the aldehyde oxidase regulatory system.

# MATERIALS AND METHODS

## General Methods

### 1 Drosophila Stocks

All Drosophila melanogaster strains used are described in Table 1. These strains were maintained in stock cultures at 19°C, on formula 4-24, Instant Drosophila Food, obtained from Carolina Biological Supply Co. On occasion, flies were grown on a yeast-agar medium.

The stock number (Mid-America Stock Cultures) of the mutant strain under study is h116. As stated previously, two of the h116's phenotypes were a low level of aldehyde oxidase activity, and a temperature sensitive lethality at 29°C. These two phenotypes were first mapped to their specific chromosomal location then characterized separately.

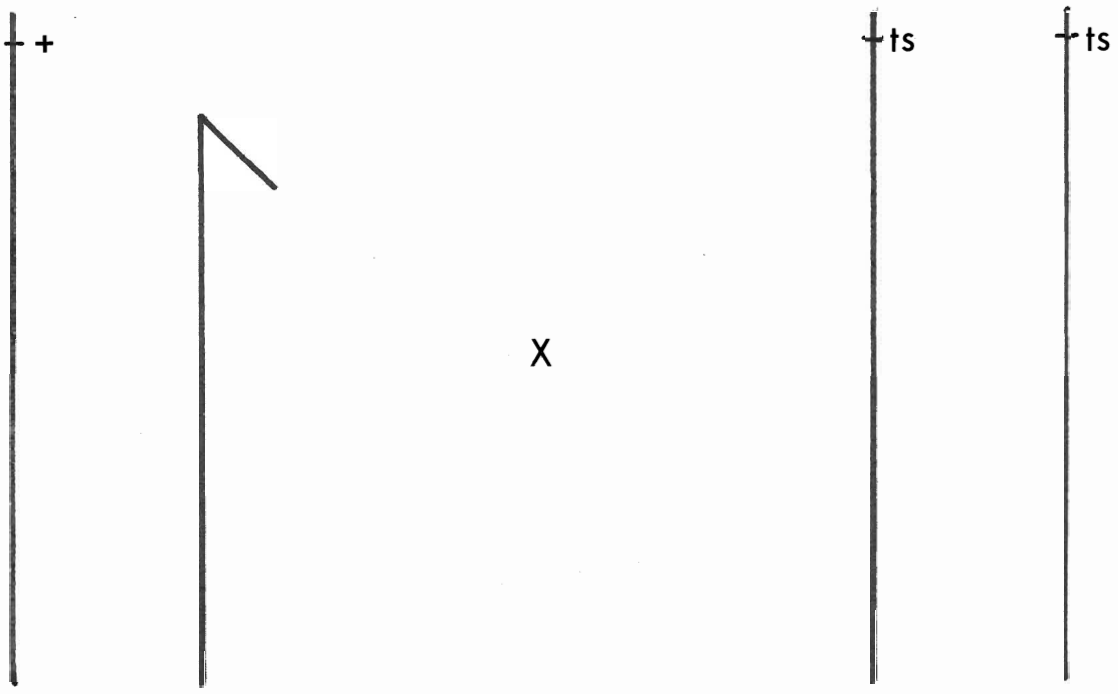
### 2 Genetic Mapping of Temperature Sensitivity

The initial cross, made to determine which chromosome the temperature sensitive lethal (ts) mutation was on, is shown in Figure 1. A homozygous h116 female

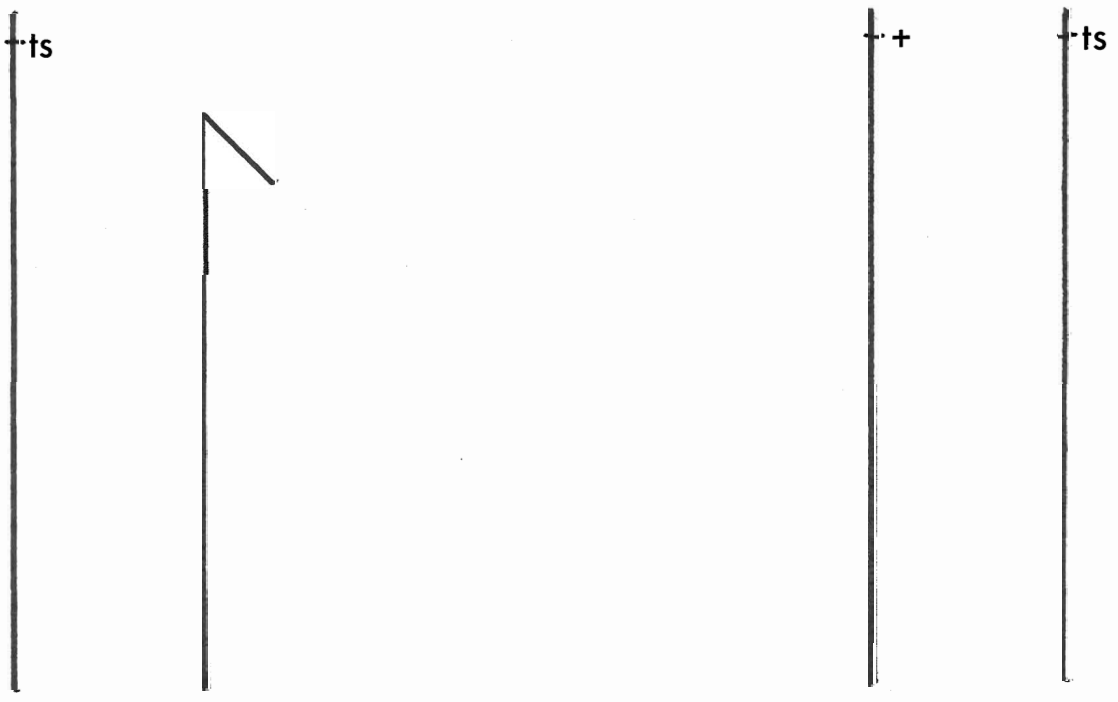
Figure 1

Figure 1 shows the cross used to determine if the temperature sensitive mutation (ts) was on the X-chromosome. $P_1$a represents a wild type male crossed to an hll6 female ($P_1$b). If the temperature sensitive mutation is on the X-chromosome, the offspring should be 50% males which are hemizygous for the ts mutation ($F_1$a), and therefore inviable at 29°C, and 50% females which are heterozygous for the ts mutation ($F_1$b), and therefore viable at 29°C.

Fig 1




P1a P1b




F1a F1b

Table 1

Table 1 is a description of all genetic stocks used, and their origin.

| Genotype/Stock No. | Description | Origin |
|---|---|---|
| hll6 | ts, ao- | Bowling Green Stock Center |
| y,w | Yellow body, White eyes | Stock Center |
| y,w//sc8 w | Males as yw, with translocation of y chromosome | Stock Center |
| ac//sc8 ac | as above, but with achaete | Stock Center |
| w-# | White recombinent | Generated in lab |
| y-# | Yellow recombinent | Generated in lab |
| w-hll6 | White recombinent | Generated in lab |
| Canton S | Wild type | Dr. R. Singh McMaster University |

was crossed to a yellow white male, at 19°C. The flies were then shifted to 29° C immediately. The progeny consisted of heterozygous females, and males, hemizygous for the hl16 chromosome. All progeny was heterozygous for the autosomes. If the ts mutation was dominant (either autosomal or sex linked), both sexes would be inviable at 29°C. If the mutation was recessive autosomal one, both sexes would be viable. If the mutation was a recessive sex linked one, only the females would be viable. The males would be hemizygous for the mutation, and therefore would be inviable. This method was used to test for temperature sensitivity throughout the study.

3 Electrophoretic Analysis of Aldehyde Oxidase Activity

Single adult flies were homogenized on ice, in 20 ul of 0.1M tris borate EDTA buffer, pH 8.9, containing 5% sucrose. These samples were electrophoresed on 6% poly-acrilamide gels, with 0.1M tris borate EDTA buffer, pH 8.9, as the running buffer. The gels were run for 2 hours at 300 volts, 100 milliamps. These gels were then removed from the electrophoresis apparatus, rinsed in distilled water, and stained for Aldehyde Oxidase activity, and in some cases, Xanthine Dehydrogenase activity as well. The staining solution for Aldehyde Oxidase consisted of, 100 ml

of o.1M tris HCl buffer, pH 8.5, 26 mg of nitro blue tetrazolium (NBT), 2ml of phenazine methosulphate (PMS) (at a concentration of 2 mg/ml) and 8 ml of benzaldehyde as a substrate. The staining solution for Xanthine Dehydrogenase was essentially the same, except 80 mg of NBT was used, and 30 mg of NAD was added. The substrate used for Xanthine Dehydrogenase was 12 ml of 0.05M Hypoxanthine in 0.1N HCl. The staining solution was added to the gel, and left for one to two hours, in the dark. After the activity bands appeared, the gel was removed from the staining solution, and fixed in a methanol-acetic acid solution.

4 Quantitave Assay of Aldehyde Oxidase Activity

A modified form of Dickinson's (1965) assay was used. Individuals were homogenized in 0.2M potassium phosphate buffer, pH 7.5. An assay reaction mixture was prepared, which consisted of 0.5M acetaldehyde, 1mg/ml of bovine serium albumin (BSA), 0.001M EDTA, 0.04 mg/ml of PMS, and 0.02 mg/ml of diclorophenol-indophenol (DCPIP) in 0.2M potassium phosphate buffer, pH 7.5.

A 100 ul sample of the homogenate was mixed with 2.5 ml of the reaction mixture, in a cuvette, and the decrease in absorbance was recorded at 600 nm on a Turner Recording Spectrophotometer.

The protein content of each homogenate was determined using Miller's modification (1966) of the Lowry procedure (Lowry et al., 1951).

## Specific Experiments

### 1 Chromosomal Mapping of the Mutations

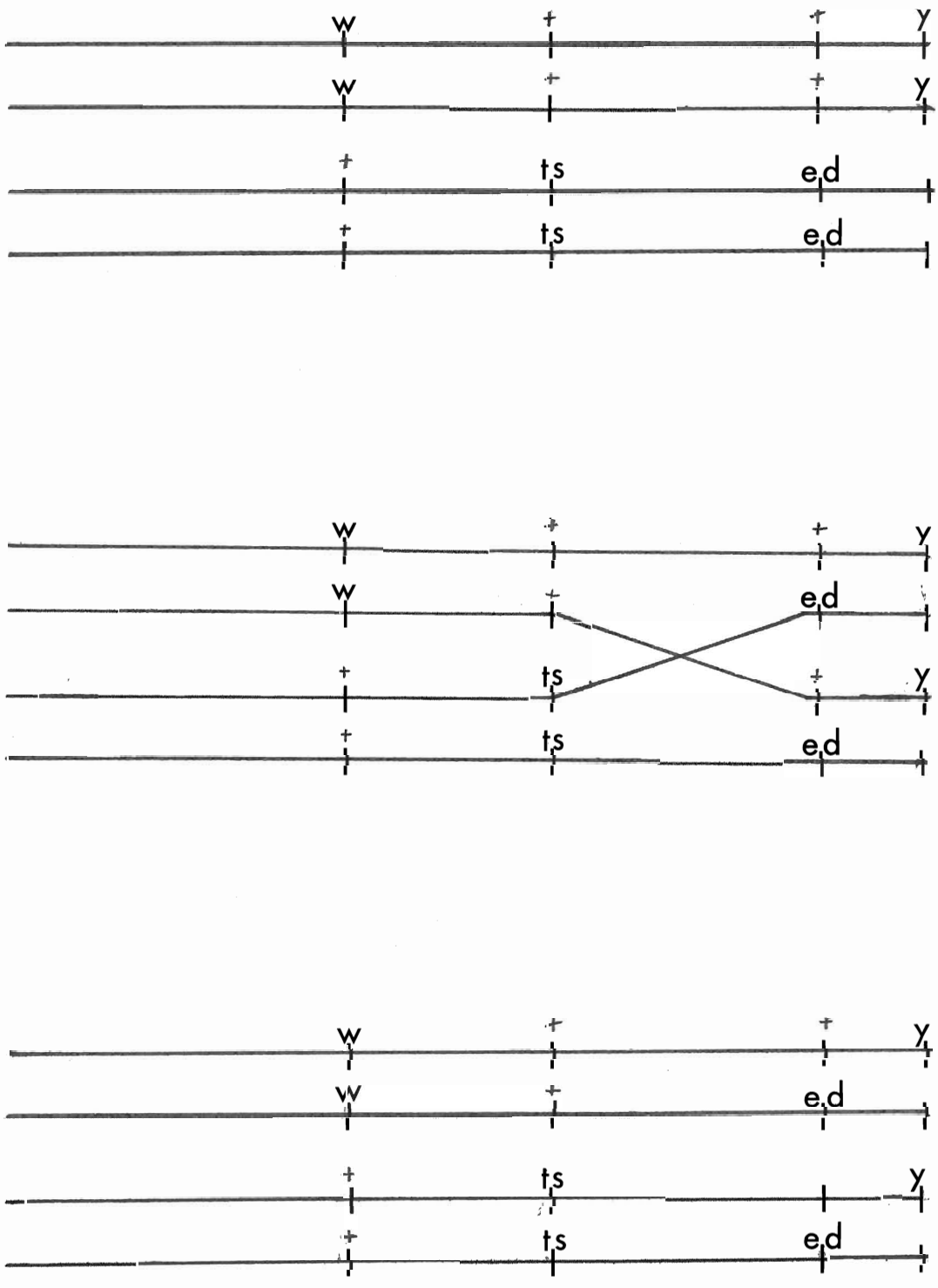
The enzyme deficiency had previously been mapped to the X chromosome by Hickey and Singh (unpublished). Therefore the first step in mapping the temperature sensitivity, was to determine whether it also mapped to the X chromosome. The method for this was described previously in the General Methods Section ( 2, pages 31,32).

Once it had been established that both mutations were on the X chromosome, the next step was to determine where they were located. This was done using a recombination analysis (Figure 2). Female hl16 virgins were crossed to yellow white males, generating yellow white/hl16 heterozygote female progeny, and male progeny hemizygous for the hl16 X chromosome. Therefore in this cross, recombination between the X chromosomes could only take place in the female progeny. These female progeny were then back crossed to the yellow white males. The male progeny of

Figure 2

Figure 2 shows a recombination event between the temperature sensitive lethal mutation (ts), and the enzyme deficiency mutation (ed), as it would occur in a female, heterozygous for the h116 (ts, ed) and yellow-white (y w) x-chromosomes. Wild type alleles of the mutations are shown as + .

Fig 2



this second cross were hemizygous for either the hll6 X chromosome, or yellow white X chromosome. The female progeny were either homozygous for the yellow white X chromosome, or heterozygous for the two X chromosomes.

When a recombination event occured between the yellow and white loci in the heterozygous female Fl progeny, the recombinant progeny's phenotype was either yellow only or white only. The F2 males, being hemizygous for the single market mutation, would express it. The females would be homozygous for one of the market mutations, and heterozygous for the other mutation. Therefore, only one marker mutation would be expressed (Figure 2a)

Once the recombinant progeny were obtained, the females were crossed to the yellow white males, and the male progeny of this cross which had the recombinant X chromosome, were treated like the other recombinant males.

The recombinant males were crossed to hll6 females. The Fl female progeny of this cross were heterozygous for the hll6 X chromosome, and the recombinant X chromosome. These females were then back crossed to their male parents. The F2 females of this back cross, were then either homozygous for the recombinant X chromosome, or heterozygous

for the hll6, and recombinant X chromosomes. The F2 males were hemizygous for either the X chromosomes. The progeny that were either homozygous or hemizygous for the recombinant X chromosome were then crossed with each other to generate homozygous recombinant lines (Fig 3).

Once these recombinant lines were generated, they were tested for their aldehyde oxidase activity (and in some cases their xanthine dehydrogenase activity), and temperature sensitivity. Both electrophoresis and enzyme assays were used to determine the aldehyde oxidase activity. The test for temperature sensitivity involved crossing the recombinant females to wild type males, and shifting them up to 29°C, as described in the general methods section (A-2, pages 31 and 32). After it was determined where the two mutations were located, an approximate map distance was calculated.

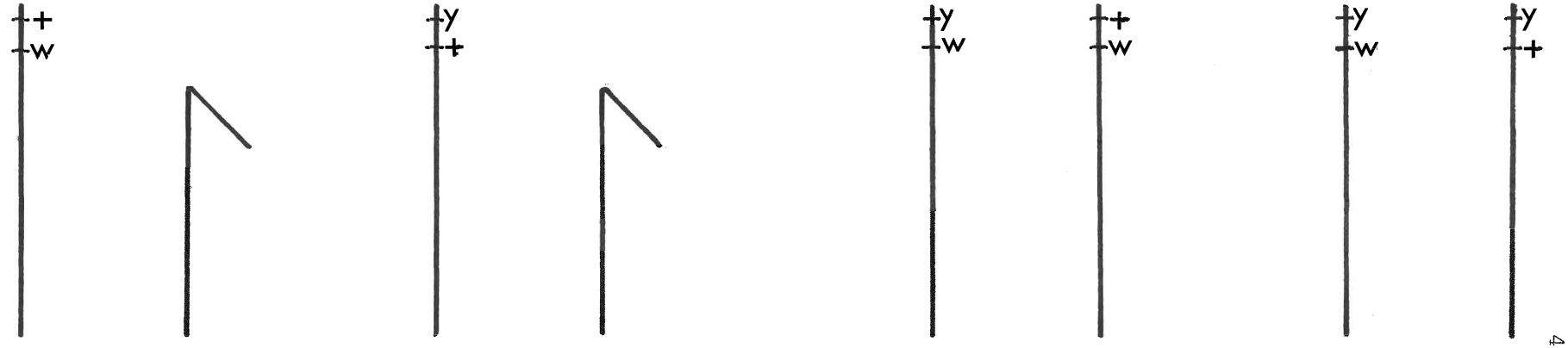
To further determine where exactly the mutations were located, and further demonstrate that they were in fact two separate mutations, female hll6 virgins were crossed to males which had a very small section of wild type X chromosome translocated on to their Y chromosome. This strain was the sc8 strain (See figure 1). This translation covered only position 0.0 of the X chromosome.

Figure 3

Figure 3 represents the cross used to recover the recombinant X-chromosome. In this cross, a yellow-white male ($P_1$a) is crossed to a female, in which a recombination event has occured ($P_1$b). The progeny of this cross are: males, hemizygous for the white mutation ($F_1$a), males, hemizygous for the yellow mutation ($F_1$b), females, homozygous for the white mutation and heterozygous for the yellow mutation ($F_1$c), and females, heterozygous for the white mutation and homozygous for the yellow mutation ($F_1$d). In all offspring which are progeny of a female in which a recombination event has occurred, only one of the marker mutations is expressed.

Fig 3


P1a P1b




F1a F1b F1c F1d

If a yellow white female were crossed to a sc8 male, the F1 male progeny would only express the white mutation. The yellow mutation would in fact be heterozygous due to the small piece of wild type X chromosome translocated to the Y chromosome. The white mutation would still be expressed, because, the translocation did not cover the white locus region of the X chromosome. The same reasoning was used to determine whether the translocation would cover both of the mutations in the h116 strain. It was known that the translocation covered the enzyme deficiency, because it was used in maintaining a stock carrying the cinnamon mutation, which mapped to the same position as the yellow mutation. Therefore, the cross was made to determine whether the translocation also covered the temperature sensitive mutation. Female h116 virgins were crossed to the sc8 males, at 19°C, and then shifted to 29°. The female progeny of this cross were heterozygous for the X chromosomes, and therefore viable at 29°C. The male progeny carried the h116 X chromosome, and the sc8 Y chromosome. If the translocation covered the temperature sensitive lethal mutation, the males would be viable at 29°C. If however the translocation didn't cover the temperature sensitive lethal mutation, the males would not be viable at 29°C. Since the absence of males would be expected at 29°C regardless of what the h116 females were mated to (Providing that

the males didn't have a translocation covering the temperature sensitive region), this cross was repeated at 19°C and the males were electrophoresed. This ensured that the sc8 males still carried the translocation. by the males having wild type enzyme activity. To further ensure that the sc8 males still carried the translocation, they were crossed to yellow white females, and the male progeny were examined to determine whether both yellow and white mutations were expressed.

2 Characterization of the hl16 Aldehyde Oxidase Activity

The hl16 mutant was first electrophoresed, along with wild type standards, (Canton-S, and yellow, white) to give a direct visual comparison of the aldehyde oxidase activity of both types. The xanthine dehydrogenase activity was also stained for, to determine if it was also affected by this mutation.

For a more accurate comparison of the aldehyde oxidase activity of the wild type and the hl16 type, the specific activities of each were determined using the spectrophotometric method already described ( 4, page 33,34).

This specific activity was compared to that of several other strains. The genotypes, yellow white,

Canton-S, Cal Tech-hll6, w-hll6, w-3, mal, cinnamon, and cinnamon/hll6 heterozygotes were used for this comparison. (See table 1 for description of the genotypes). Five males, and five females of each genotype were aged for five days, at 19°C, on Instant Drosophila Medium. These were then homogenized and assayed for their enzyme activities. Three replicates for each strain were made.

3 Developmental Changes in Enzyme Activity

The strains yellow white, and hll6 were used in this assay. Females were mated to males of their own strain, then allowed to lay eggs in petri dishes, containing a yeast-agar medium. These adults were then removed, and the cultures were allowed to develop. At the appropriate times, various stages were collected, homogenized, and stored at -20°C. The stages collected were egg, 1st, 2nd, and 3rd instar larvae, pupae, and adult. The concentrations of each homogenate were, egg-50/300ul, 1st instar-30/300 ul, 2nd instar, and 3rd instar-10/ml, pupae and adult-10/ml. For the adults, five males, and five females were used. Three replicates were made for each stage. These were then assayed as previously mentioned (Section 4).

## 4 Temperature Sensitivity of Aldehyde Oxidase Activity

The strains Canton-S, yellow white, cal tech-hll6, and w-3 were used. These strains were raised at 19°C. Adults, twenty four hours old or younger, were placed on new media (Instant Drosophila Food), and half the adults were shifted to 29°C, and half were left at 19°C. These were collected after two days and eight days. The concentration of the homogenates were five males and five females per ml. Three replicates of each were made.

## 5 Comparison of Aldehyde Oxidase Activity of Heterozygotes and Homozygotes

Strains with different levels of enzyme activity were crossed, and the activity of aldehyde oxidase in the heterozygotes were compared with the activity of the parental homozygotes. Crosses were made to generate the following heterozygotes;cin//hll6, cin//cs, cin//y,w, hll6//cs, cal tech-hll6//hll6, cs//y,w. These were compared with the homozygotes, cal tech-hll6, hll6, yellow white, and Canton-S. These individuals were aged for three days at 19°C, then assayed.

## 6 Temperature Sensitivity of Viability

The period of temperature sensitivity was determined

using the method of Suzuki(1972). This method involved the use of "Shift Up and Shift Down" experiments. Preliminary results had shown that the hl16 individuals could survive to the third instar larvae stage at 29°C. These experiments were designed to show what exactly the lethal period of the hl16's life cycle was.

The shift up experiment was used to determine the end of the lethal period of the temperature sensitivity. This was determined by an increase in the survivorship of the hl16 indivuduals. For the shift up experiment, twenty five eggs of the yellow white strain, and twenty five eggs of the hl16 strain were transferred into a single vial containing the yeast-agar medium. These eggs had been laid at 19°C, on a petri dish containing carpenter's medium. One hundred of each of these vials, containing twenty five eggs of each strain were made. These were randomly divided into five experimental groups, each group containing twenty vials. The first group of vials were allowed to develop to the third instar stage, at 19°C, then half of these were shifted up to 29°C and half were left at 19°C. The second group was allowed to develop to the early pupae stage, then half the vials were shifted up to 29°C, as in the previous group, and half were left at 19°C. The third group were left to develop to the adult stage, then the adults were transferred to new food, and shifted. The fifth group was left at 19°C as a control.

The shift down experiment allowed the beginning of the sensitivity period to be determined, which was seen as a decrease in survivorship of the hl16 strain. For this experiment, twenty five eggs of the yellow white strain, and twenty five eggs of the hl16 strain were transferred to a single vial containing the yeast-agar medium as was the case in the shift up experiment. However, these eggs were laid at 29°C on a petri dish containing the yeast-agar medium. A total of ninety six of these vials, containing twenty five eggs of each strain were made. These were randomly divided into six groups, within sixteen vials in each group. Half of the vials in each group were shifted to 19°C immediately, and half were left at 29°C. The second group was allowed to develop for one day, at 29°C, then half of the vials were shifted down to 19°C, and half were left at 29°C. The third group was left at 29°C for two days, then shifted in the same manner. The fourth group was left for four days, then shifted, the fifth group was shifted on day eight, and the sixth group was left at 29°C as a control.

# RESULTS

Overall, the results showed that there were two mutations responsible for the two phenotypes seen. The mutation responsible for the enzyme deficiency mapped to position 0.0 on the X-chromosome, and the mutation responsible for the temperature sensitivity, mapped extremely close by, but was separable by recombination. The estimated map distance between the enzyme deficiency and the temperature sensitive mutation was 0.1 map units (centimorgans). The results of specific experiments were as follows:

## 1. Genetic Mapping

Genetic mapping included both the assignment of mutations to chromosomes and the location of the mutation within a chromosome.

### 1.a. Chromosomal Assignments

Separate experiments were performed for the two phenotypic effects: enzyme deficiency and temperature sensitivity of viability.

#### 1.a.1. Enzyme Deficiency

It was found that the aldehyde oxidase deficiency mutation mapped to the X-chromosome. This was determined

by comparing the aldehyde oxidase activity of male and female progeny of homogygous hll6 females, which had been mated to males with wild type enzyme activity (yellow white). The results of this cross are shown in Figure 4. Females which are heterozygous for the X-chromosome showed aldehyde oxidase activity close to that of the wild type controls. Males which were hemizygous for the X-chromosome showed low enzyme activity, indistinguishable from that of the hll6 controls.

1.a.2. Temperature Sensitivity

The temperature sensitivity was also found to map to the X-chromosome. This was determined by crossing an hll6 female to a wild type male, and looking at the ratio of male to female progeny, as described in the materials and methods section. When this cross was made, the results were as follows: at 29°C, 84 female progeny developed, and no male progeny survived. At 19°C, there were 172 female progeny, and 131 male progeny. These results demonstrate that the temperature sensitivity as well as the enzyme deficiency maps to the X chromosome.

1. b. Chromosomal Localization of Mutations

Once it had been established that both aspects of the phenotype mapped to the X-chromosome, the next step was to determine where on the chromosome these loci were

Figure 4

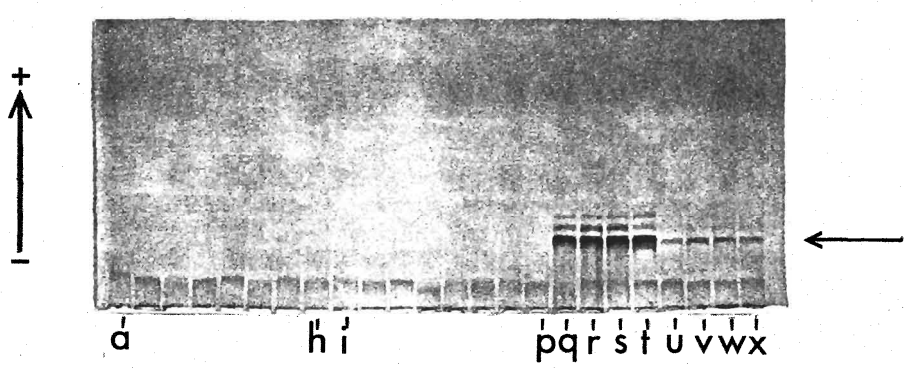
Figure 4 shows an electrophoretic gel stained for aldehyde oxidase activity.

Samples in pockets are:

| | | |
|---|---|---|
| a - h | w-hll6 female recombinant | raised at 29°C |
| i - p | w-hll6 male recombinant | raised at 29°C |
| q, r | Canton S female | raised at 19°C |
| s, t | Canton S male | raised at 19°C |
| u, v | hll6 female | raised at 19°C |
| w, x | hll6 male | raised at 19°C |

Position of major band and running direction are indicated.

Fig 4



located. It was found that the mutations mapped to the yellow region of the X-chromosome, at position, 0.0. This was determined using a recombination analysis, described in the materials and methods section.

Recombinants were recovered in the yellow-white region of the X-chromosome (map position 0.0 to 1.5). A total of fifty one strains were generated, twenty eight of which were recombinants which carried the yellow mutation, and twenty three of which were recombinants which carried the white mutation. All recombinants were then tested for aldehyde oxidase activity, and temperature sensitivity. Figure 5 shows the aldehyde oxidase and Xanthine dehydrogenase electrophoretic banding pattern and activity for these strains. All white recombinant strains showed the enzyme deficiency. The yellow recombinants showed the wild type enzyme activity. This indicated that the enzyme deficiency was inseparable from the yellow locus (map position 0.0).

All recombinant strains were also tested for their temperature sensitivity. This was done by crossing recombinant females to wild type males, and determining the sex ratio of the progeny which develop at 29°C. These results are shown in Table 2. All white recombinants were temperature sensitive, and all yellow-recombinant strains, except one, were not. The yellow 7 strain turned

Table 2

Table 2 shows the results of the recombination study, testing the recombinant lines for aldehyde oxidase activity (A.O.Activity), and temperature sensitivity (TS). The + sign refers to the wild type phenotype, and the - sign refers to the mutant phenotype.

| Strain No. | A.O.Activity | TS | Strain No. | A.O.Activity | TS |
|---|---|---|---|---|---|
| yw | + | + | h116 | - | - |
| y-18 | + | + | w-3 | - | - |
| y-5 | + | + | w-19 | - | - |
| y-II | + | + | w-18 | - | - |
| y-23 | + | + | w-8 | - | - |
| y-2 | + | + | w-5 | - | - |
| y-D | + | + | w-I | - | - |
| y-A | + | + | w-22 | - | - |
| y-19 | + | + | w-14 | - | - |
| y-20 | + | + | w-23 | - | - |
| y-34 | + | + | w-17 | - | - |
| y-39 | + | + | w-11 | - | - |
| y-30 | + | + | w-12 | - | - |
| y-9 | + | + | w-16 | - | - |
| y-14 | + | + | w-III | - | - |
| y-4 | + | + | w-2 | - | - |
| y-C | + | + | w-1 | - | - |
| y-I | + | + | w-3 | - | - |
| y-7 | + | - | w-13 | - | - |
| y-28 | + | + | w-4 | - | - |
| y-26 | + | + | w-10 | - | - |
| y-22 | + | + | w-15 | - | - |
| y-31 | + | + | w-II | - | - |
| y-13 | + | + | | | |
| y-41 | + | + | | | |
| y-3 | + | + | | | |
| y-33 | + | + | | | |
| y-11 | + | + | | | |

Figure 5

Figure 5 shows an electrophoretic gel stained for both aldehyde oxidase and xanthine dehydrogenase.

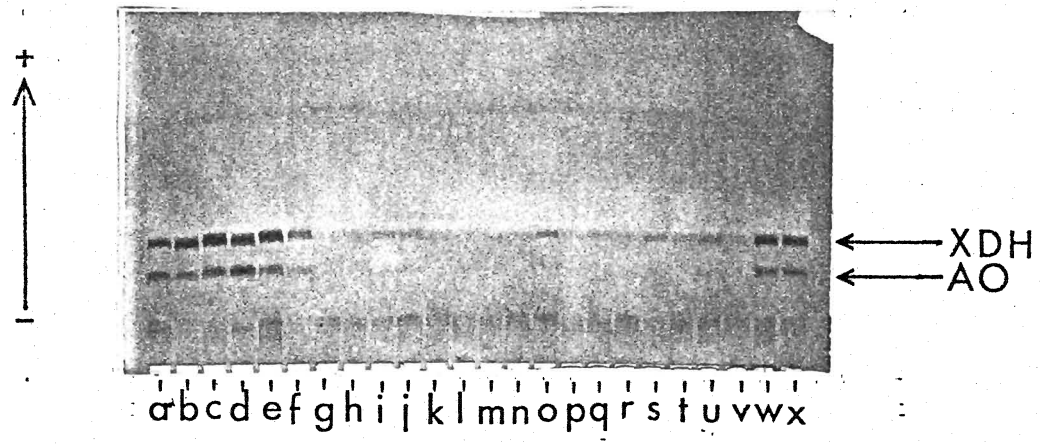
Samples in pockets are:

| | |
|---|---|
| a - f | yellow male recombinant |
| g - t | white male recombinant |
| u | hll6 female |
| v | hll6 male |
| w | y,w female |
| x | y,w male |

Positions of major bands, and running direction are indicated.

Fig 5





out to be temperature sensitive. This indicated that the two phenotypes were due to two separate mutations, which map extremely close to each other, and not due to a single mutation which affected both phenotypes (see Appendix I for numbers of males and female progeny of the crosses).

To further demonstrate that these two phenotypes were due to two separate mutations, both of which map to, or near position 0.0 on the X-chromosome, an h116 female was crossed to a male carrying a small section of the X-chromosome translocated to the Y-chromosome (see materials and methods section 1.a.). When this cross was made, and the progeny allowed to develop at 29°C, 109 female progeny were recovered. No male progeny were recovered. This indicated that the translocation did not complemennt the temperature sensitivity, which means that it lay outside the region. This cross was repeated using the sc8/ac3 strain, and the developing cultures were allowed to develop both at 19°C and at 29°C. At the higher temperature, no males developed, but 94 females eclosed. At the lower temperature, 123 females, and 127 males eclosed.

The males which eclosed from this cross at 19°C were electrophoresed and stained for their aldehyde oxidase

activity. This was done to confirm that the males still had the translocation. The sc8 males were also crossed to yellow white females to further demonstrate that the translocation was still present. The results for the electrophoresis are shown in Figure 6. The sc8 males did have wild type enzyme activity. The results of the cross to the yellow white females were 96 wild type females, and 81 white eyed males. This demonstrated that there was complementation for both the enzyme deficiency, and the yellow mutation. Therefore, these results show that there are two separate mutations responsible for the temperature sensitivity and the enzyme deficiency, and that the enzyme deficiency maps to the yellow locus at position 0.0, with the temperature sensitivity close by.

1. c. Complementation Study

1.c.1.Complementation Between cin and h116

Once the mutations controlling the enzyme deficiency and the temperature sensitivity had been mapped, females which were heterozygous for the cin and h116 X-chromosomes were constructed to determine whether the enzyme deficiency mutation could complement the cin mutation. The results for this are shown in Figure 6. These heterozygotes showed complementation for the aldehyde oxidase activity,

Figure 6

Figure 6 shows an electrophoretic gel, stained for aldehyde oxidase. Samples in pockets are:

a - i cinnamon females

j - m cinnamon//hll6 heterozygote females

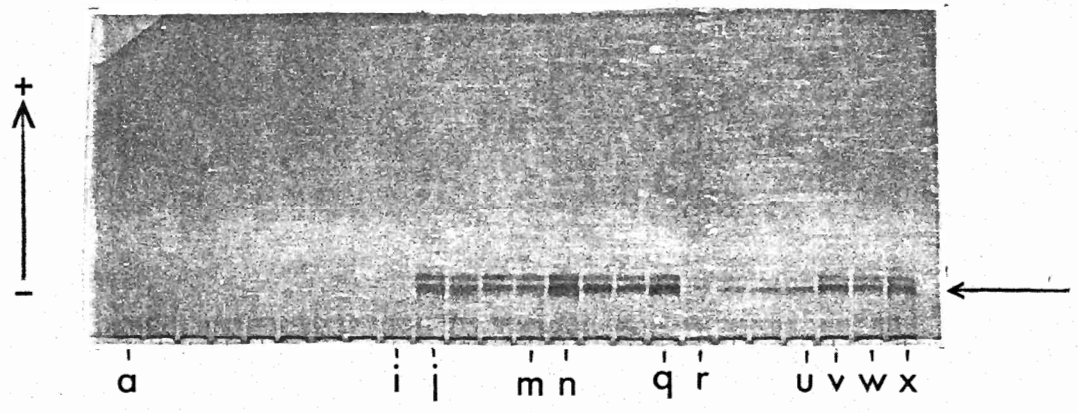
n - q hll6//$Sc^8$ heterozygote males

r - u hll6 females

v - x Canton S females

Position of major band and running direction are indicated.

Fig 6



showing an activity close to that of wild type, thus indicating that the hll6 mutation is a separate mutation from that of the cin mutation (Figure 7). The activity of the heterozygotes was quantified and found to be somewhat less than the activity of the wild type (Table 3). To determine whether this was due to incomplete complementation, or if it was due to an effect of dosage compensation, heterozygotes of the types hll6//canton s, hll6// yellow white, hll6//cal tech, hll6//cin and cin//sc8 were constructed, and their activity was determined. The results of this are seen in Table 4. It can be clearly seen that there was no difference in activity between the hll6//wild type heterozygotes and the wild type homozygotes. However, there was still a decreased activity seen in the hll6//cin heterozygotes. The low activity of the hll6//cal tech heterozygote confirmed that these two had the same mutation for the enzyme deficiency.

2. Characterization of the Enzyme Deficiency

The aldehyde oxidase activities of the hll6 strain, and the yellow white strain, as shown on an electrophoretic gel can be seen in Figure 4. To further quantify the differences in activities between the two, the

Table 3

Table 3 shows the aldehyde oxidase activity (change in absorbance (abs)/minute (min)/ g of protein) for various genotypes.

See text for description of assay conditions.

Comparison of Genotypes

| Genotype | Aldehyde Oxidase Activity | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | $\bar{X}$ | S.E. |
| hll6 | 0.37 | 0.69 | 0.74 | 0.60 | 0.16 |
| Cal Tech | 0.42 | 0.65 | 0.39 | 0.49 | 0.12 |
| Canton S | 4.2 | 3.5 | 3.5 | 3.6 | 0.29 |
| y,w | 7.5 | 6.6 | 5.4 | 6.5 | 0.86 |
| l mal | 0 | 0 | 0 | 0 | - |
| cin | 0 | 0 | 0 | 0 | - |
| cin//hll6 | 2.2 | 2.7 | 3.4 | 2.8 | 0.49 |

Figure 7

Figure 7 shows aldehyde oxidase activities in males, hemizygous for the enzyme deficiency mutation, and females, heterozygous for the enzyme deficiency mutation and the wild type allele.

The samples in the pockets are:

| | |
|---|---|
| a | w II male |
| b | w l9 male |
| c | w-l3 male |
| d | w I male |
| e, f | w II//wild type female |
| g, h | w II male |
| i, j | w l9//wild type female |
| k, l | w l9 male |
| m, n | w l3//wild type female |
| o, p | w l3 male |
| q, r | w I//wild type female |
| s, t | w I male |
| u | wild type female |
| v | wild type male |
| w | hll6 female |
| x | hll6 male |

Fig 7

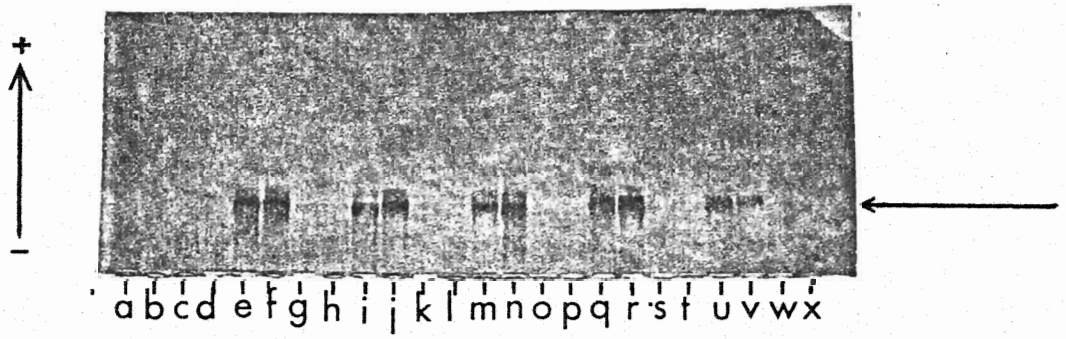


Table 4

Table 4 shows the aldehyde oxidase activity (change in absorbance/minute/ g of protein).

Note--all flies used were females, except for the assays involving the sc8 translocation.

Comparison of Heterozygotes and Homozygotes

| Genotype | Aldehyde Oxidase Activity | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | $\bar{X}$ | S.E. |
| cin//hll6 | 2.9 | 4.2 | 3.9 | 3.7 | 0.56 |
| cin//Canton S | 3.1 | 5.9 | 6.1 | 5.0 | 1.3 |
| cin//y,w | 4.9 | 5.7 | 6.2 | 5.6 | 0.53 |
| hll6//Canton S | 3.1 | 4.2 | 5.3 | 4.2 | 0.90 |
| Cal Tech//hll6 | 0.42 | 0.49 | 0.32 | 0.41 | 0.069 |
| Canton S//y,w | 5.7 | 4.7 | 7.3 | 5.9 | 1.1 |
| Cal Tech | 0.39 | 0.29 | 0.58 | 0.42 | 0.12 |
| hll6 | 0.42 | 0.37 | 0.51 | 0.43 | 0.06 |
| y,w | 6.4 | 7.9 | 7.3 | 7.2 | 0.61 |
| Canton S | 3.2 | 2.1 | 4.1 | 3.1 | 0.82 |

Table 5

Table 5 shows the aldehyde oxidase activity of the recombinant lines. Activity is change in absorbance/minute/ng of protein.

| Strain No. | Activity | Strain No. | Activity |
|---|---|---|---|
| yw | 6.7 | hl16 | 0.45 |
| y-18 | 4.9 | w-3 | 1.60 |
| y-5 | 3.8 | w-18 | 0.71 |
| y-11 | 4.1 | 4-8 | 0.52 |
| y-23 | 3.1 | w-5 | 1.10 |
| y-2 | 3.9 | w-I | 0.44 |
| y-D | 2.5 | w-22 | 0.79 |
| y-11 | 4.8 | w-14 | 0.39 |
| y-25 | 4.3 | w-23 | 0.26 |
| y-A | 2.7 | w-17 | 0.00 |
| y-17 | 5.6 | w-11 | 1.50 |
| y-20 | 3.7 | w-12 | 0.55 |
| y-34 | 5.4 | w-16 | 0.00 |
| y-39 | 5.9 | w-III | 0.30 |
| y-30 | 4.9 | w-2 | 0.67 |
| y-9 | 3.3 | w-1 | 0.00 |
| y-14 | 4.6 | w-13 | 0.25 |
| y-4 | 4.9 | w-4 | 1.00 |
| y-C | 4.2 | w-3 | 0.00 |
| y-I | 3.5 | w-21 | 0.34 |
| y-7 | 3.6 | w-10 | 0.00 |
| y-28 | 5.9 | w-15 | 0.77 |
| y-26 | 3.7 | w-II | 0.53 |
| y-22 | 5.0 | | |
| y-31 | 4.0 | | |
| Mean Activity | 4.4 | Mean Activity | 0.52 |

aldehyde oxidase activities were determined spectrophotometrically (see Materials and Methods). Table 3 shows the specific activities of aldehyde oxidase of various strains of Drosophila melanogaster. (Genotypes are described in Table 1 in the Materials and Methods Section.) The two wild type strains, Canton-S and yellow white, had a much greater activity than did the hl16, and cal tech-hl16 strains. The difference ranged from six to twelve fold. The strains, mal and cin showed no detectable activity in this assay. The cin//hl16 heterozygous females showed aldehyde oxidase activity close to that of wild type strains.

To further quantify the differences between wild type activity and the enzyme deficiency, the recombinant lines were assayed and compared to that of the hl16 and wild type activities. The results are shown in Table 5. There was virtually no difference between the hl16 activity and that of the white recombinants, nor was there any difference between the activity of the yellow white strain, and the yellow recombinants. Combining the low enzyme activity of the white recombinants, and the hl16 strain, and comparing it with the high enzyme activity of the yellow white recombinant strain and the yellow shite strain, it was determined that on average the enzyme deficient strains had about 12% of the aldehyde oxidase activity of that of the wild type.

2.a. The Developmental Profile of the hl16 Aldehyde Oxidale Activity as Compared to that of the Wild Type Enzyme Activity.

The results of the developmental profiles for the wild type and hl16 strains are shown in Table 6, and in Figure 8. It can be seen that in the yellow white strain, the activity is not detectable until the second instar larvae stage. At this stage, the enzyme activity reached a maximum, and slowly decreased to the adult stage. In the hl16 strain, the enzyme activity was not detectable until the third instar larvae stage, and then remained low throughout its development, decreasing as the flies developed to adults.

Figure 4 shows the enzyme activity of the w-hl16 strain raised at 29°C, as shown on an electrophoresis gel. There was no detectable activity for the aldehyde oxidase. This suggested that the aldehyde oxidase enzyme itself was temperature sensitive, becoming totally inactive at the higher temperatures. To determine exactly the differences in aldehyde oxidase activity in flies raised at the two temperatures, various strains were raised at 19°C and 29°C (as described in the materials and methods section), and assayed on the Turner recording spectrophotometer. The results for this experiment are

Table 6

Developmental Assay

Table 6 shows the comparison in Aldehyde Oxidase Activity over time in the yellow, white, and hll6 strains.

| Developmental Stage | Activity | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | y.w | | | $\bar{X}$ | SE | hll6 | | | $\bar{X}$ | SE |
| Egg | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1st Instar | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2nd Instar | 3.3 | 5.7 | - | 4.5 | 1.7 | 0 | 0 | 0 | 0 | 0 |
| 3rd Instar | 4.6 | 3.4 | 3.8 | 3.9 | 0.61 | 1.7 | 0.67 | 0.64 | 1.0 | 0.6 |
| Pupae | 4.0 | 2.7 | 2.0 | 2.9 | 0.92 | 0.25 | 0.16 | - | 0.20 | 0.06 |
| Adult | 1.2 | 1.3 | 1.4 | 1.3 | 0.10 | 0.21 | 0.25 | 0.18 | 0.21 | 0.35 |

Figure 8

Figure 8 shows the comparison of aldehyde oxidase activities between the yellow-white strain (open circle), and the hll6 strain (closed circle) over various stages of development.

These stages are: lst, 2nd, and 3rd larval stages (L-l, L-2, L-3), pupae (P), and adult (A).

A. O. activity is in change in absorbance/min/ g of protein.

Fig 8

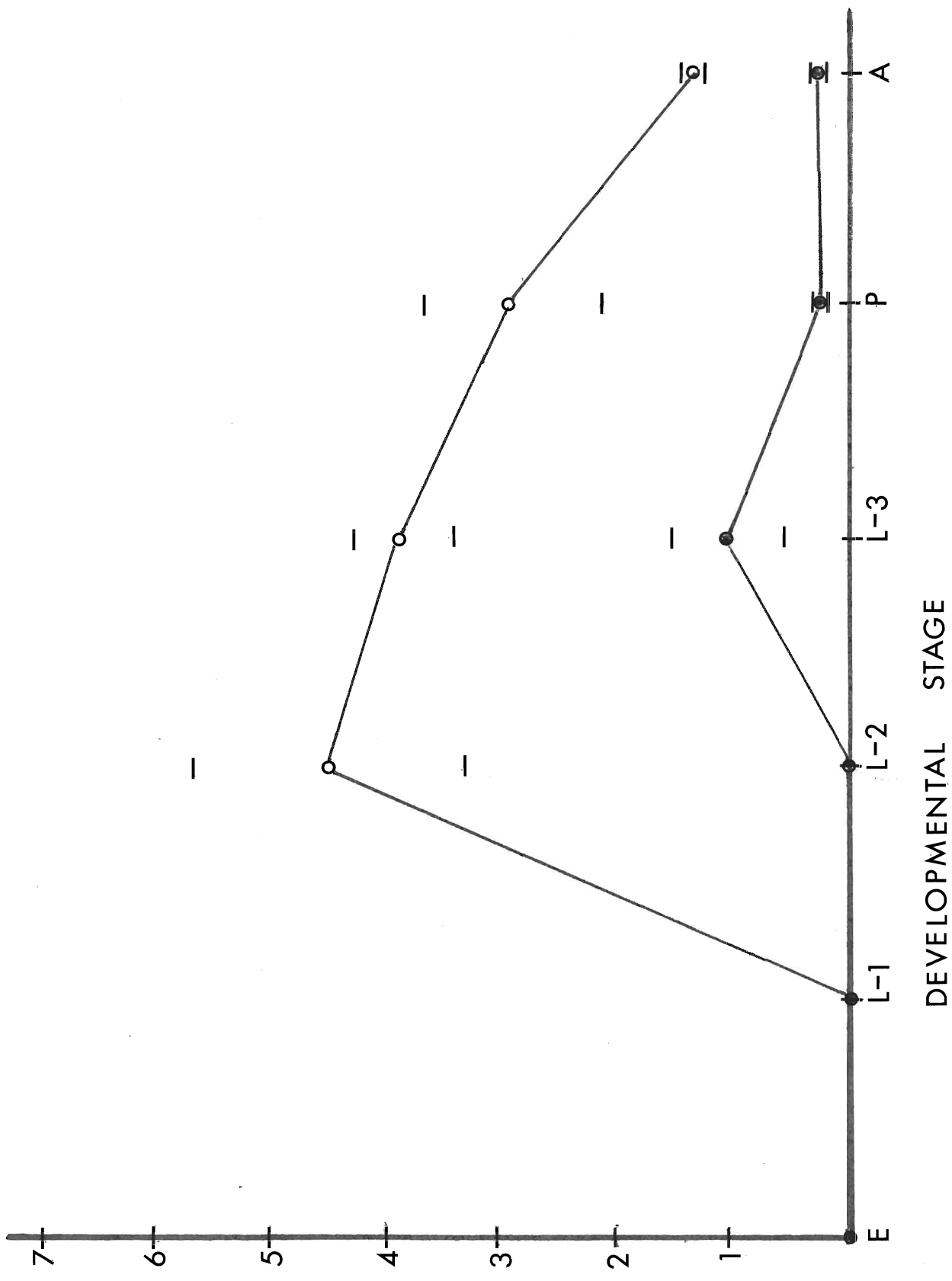


Table 7

Table 7 shows the aldehyde oxidase activity (change in absorbance/minute/ng of protein) for various strains at 19°C, 29°C after 2 days and 29°C after 8 days. The h116, w-3 and Cal Tech strains showed zero activity at 29°C.

Temperature Sensitivity of Aldehyde Oxidase Activity

| Strain | Aldehyde Oxidase Activity | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 19°C | | | | | 29°C (2 days) | | | | | 29°C (8 days) | | | | |
| | 1 | 2 | 3 | $\bar{X}$ | S.E. | 1 | 2 | 3 | $\bar{X}$ | S.E. | 1 | 2 | 3 | $\bar{X}$ | S.E. |
| y,w | 5.3 | 4.9 | 5.7 | 5.3 | 0.33 | 4.3 | 4.9 | 5.4 | 4.8 | 0.45 | 4.2 | 3.7 | 4.2 | 4.0 | 0.24 |
| Canton S | 2.9 | 3.1 | 6.4 | 4.13 | 1.6 | 2.1 | 3.2 | 4.9 | 3.4 | 1.15 | 3.2 | 2.8 | 2.7 | 2.9 | 0.22 |
| w-h116 | 0.39 | 0.30 | 0.10 | 0.26 | 0.12 | 0 | 0 | 0 | 0 | - | 0 | 0 | 0 | 0 | - |
| w-3 | 0.21 | 0.10 | 0.31 | 0.23 | 0.11 | 0 | 0 | 0 | 0 | - | 0 | 0 | 0 | 0 | - |
| Cal Tech | 0.43 | 0.30 | 0.42 | 0.38 | 0.05 | 0 | 0 | 0 | 0 | - | 0 | 0 | 0 | 0 | - |

shown in Table 7. It can be clearly seen that there is a marked effect on the enzume activity of the hll6 strain raised at 29°C. There is no detectable activity for the indivuduals at the higher temperature, while the aldehyde oxidase activity for the wild type strains is not affected.

3. Characterization of the Temperature Sensitive Lethality

The characterization of the temperature sensitivity involved determining whether the sensitivity to temperature acted at a specific stage in the developmental period, or whether it acted throughout the life cycle. This was determined by the use of shift up and shift down experiments (see materials and methods section). Table 8 shows the results of the shift down experiment. From this experiment, it was found that the beginning of the sensitive period was in the egg stage. Even eggs which had been laid at 29°C and immediately shifted down to 19°C were sensitive. This is also illustrated in Figure 9.

The shift up experiment was designed to determine the end of the lethal period, by the increase in the survivorship of the flies. Table 9 shows the results of this experiment. From these results, it can be seen that the adult stage is the stage in which there is an increase in survivorship, this indicating the end of the

Table 8

Table 8 shows the results of the shift down experiment. Eggs were collected at 29°C and shifted to 19°C at the appropriate times.

Survivorship is calculated as number of individuals eclosing divided by the number of eggs in culture times(x) 100.

| Stage Shifted 29°C to 19°C | % Survivorship | |
|---|---|---|
| | yw | hll6 |
| Egg | 44 | 0 |
| 1st instar larvae | 48 | 0 |
| 2nd instar larvae | 70.4 | 0 |
| 3rd instar larvae | 57 | 0 |
| Pupae | 49 | 0 |
| Not shifted | 42 | 0 |

lethal period. However, the survivorship of the h116 adult still did not come close to that of the yellow white controls, therefore indicating that they still were more sensitive to temperature than the wild type. This is also illustrated in Figure 10.

Therefore these results demonstrate that the h116 strain was sensitive to temperature throughout the life cycle, with the sensitivity decreasing in the adult stage, although never becoming as resistant to high temperature as the wild type controls.

Figure 9

Figure 9 shows the comparison in survivorship of the hll6 strain (closed circle) and the Canton S strain (open circle), in the shift down experiment. The developmental stages are: 1st, 2nd, and 3rd larval stages (L-1, 1-2, L-3), pupae (P), and adult (A).

Percent survivorship is calculated as total number of individuals eclosing at 19°C over total number of eggs placed in each culture, multiplied by 100.

Fig 9

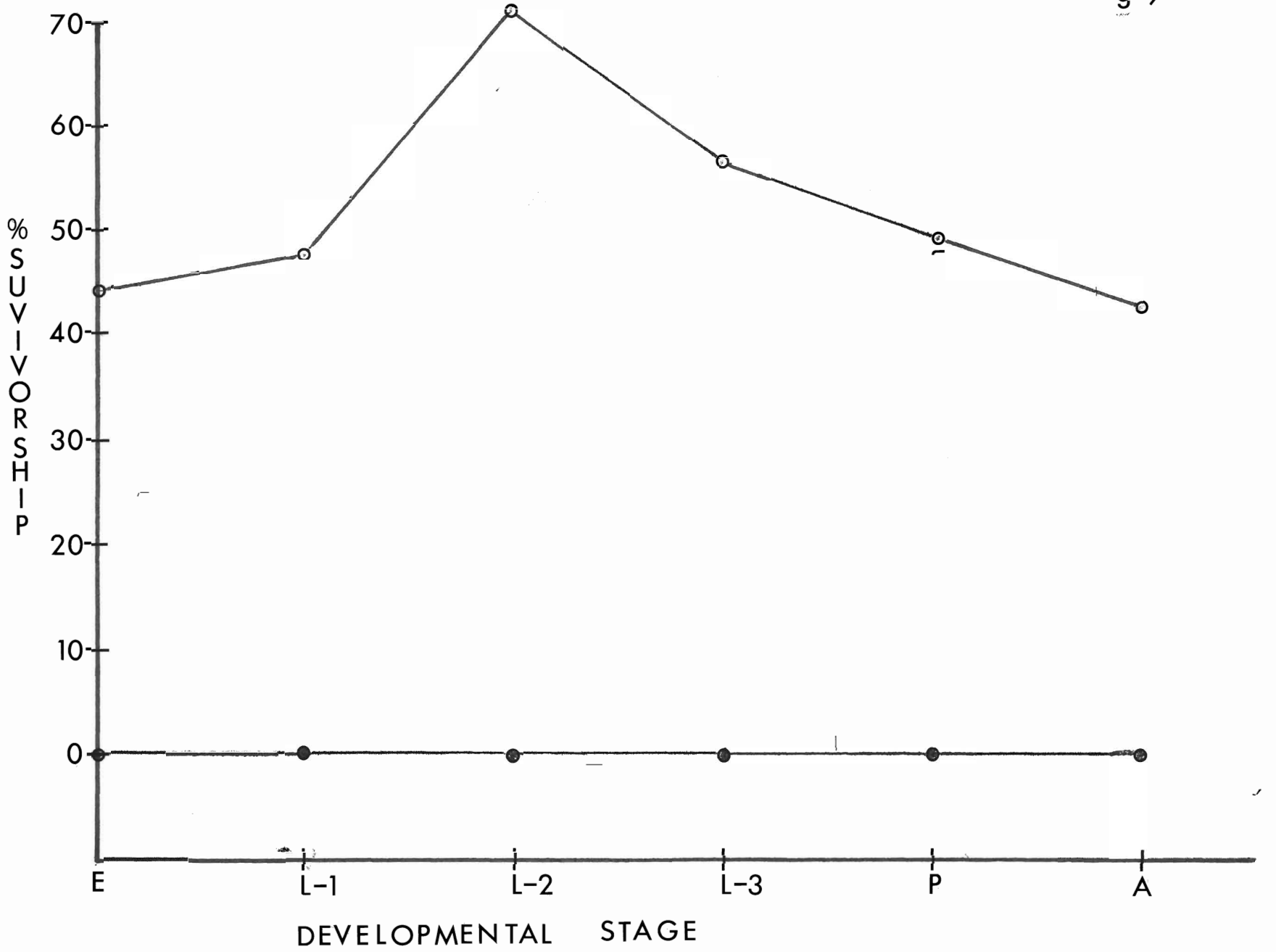

70
60
50
40
30
20
10
0
% SURVIVORSHIP
E
L-1
L-2
L-3
P
A
DEVELOPMENTAL STAGE

Table 9

Table 9 shows the results of the shift up experiment. Eggs were collected at 19°C and shifted to 29°C at the appropriate stages. Percent survivorship is calculated as number of individuals eclosing (or surviving after one week at 29°C in the case of the adults which were shifted) divided by the number of eggs in culture vials times (x) 100.

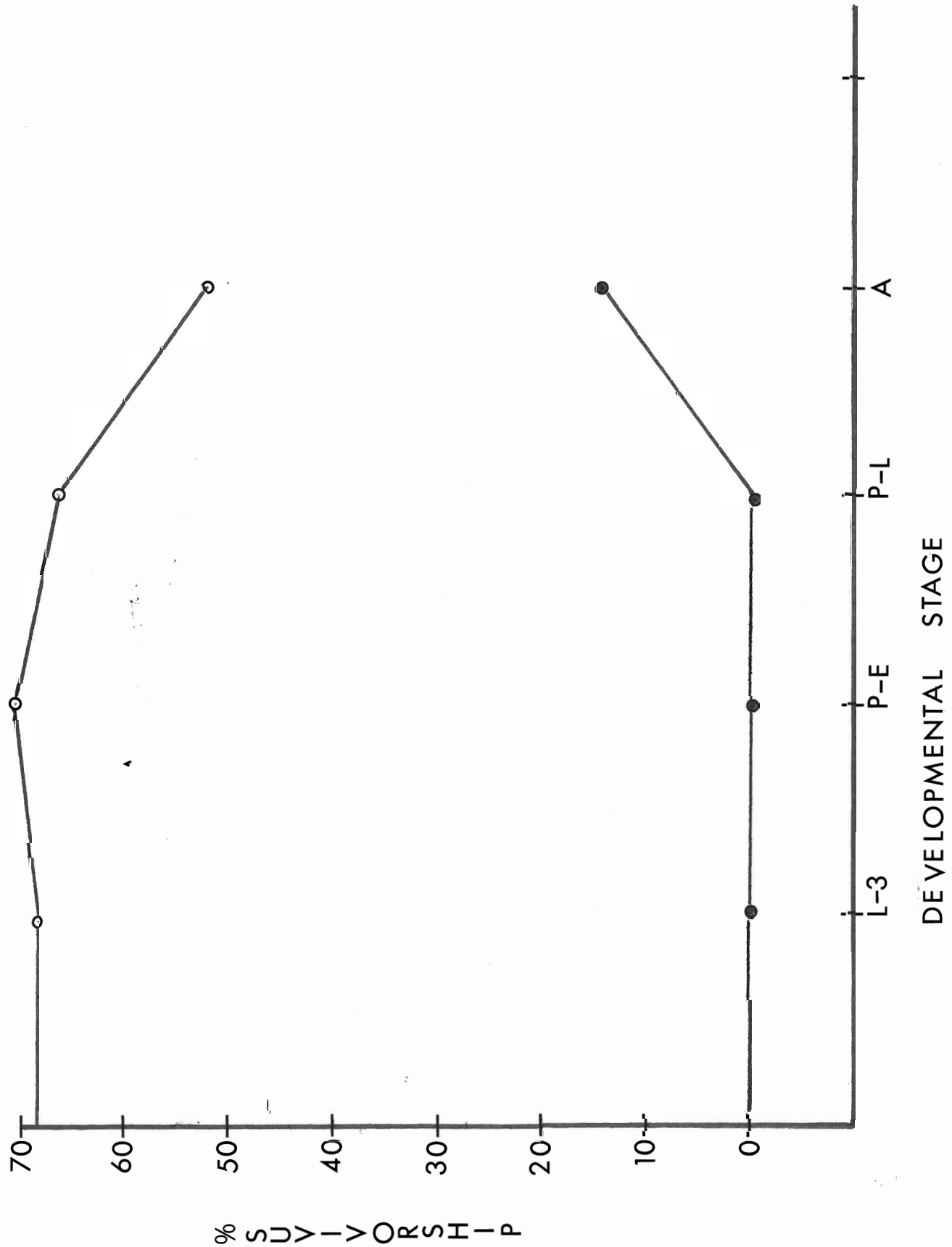
| Stage Shifted 19°C to 29°C | % Survivorship yw | hl16 |
|---|---|---|
| 3rd instar larvae | 68 | 0 |
| Early Pupae | 70 | 0 |
| Late Pupae | 66 | 0 |
| 1 week old Adult | 52 | 14 |
| Not shifted | 39 | 15 |

# Figure 10

Figure 10 shows the comparison in survivorship of the h116 strain (closed circle) and Canton S strain (open circle) in the shift up experiment. The developmental stages are 3rd instar larvae (L-3), early pupae ($P_E$), late pupae ($P_L$), and adult (A).

Survivorship is calculated as the total number of individuals surviving after one week at 29°C, over the total number of eggs placed in the culture, multiplied by 100.

Fig 10



# DISCUSSION

## 1. Introduction

One of the most extensively studied eukaryotic gene regulatory systems, is the system of genes controlling the enzymes aldehyde oxidase, xanthine dehydrogenase, and pyridoxal oxidase, in Drosophila melanogaster. This system is composed of at least seven genes, three structural genes and four regulatory genes. The three structural genes are known as the rosy locus (xanthine dehydrogenase structural gene, map position 3-52), aldox (aldehyde oxidase structural gene, map position 3-57), and lpo (pyridoxal oxidase structural gene, map position 3-57). In addition to these three structural genes, there are four regulatory genes. These are maroon-like (map position 1-64.8), which is characterized by an absence of any of these three enzymes, low xanthine dehydrogenase (map position 3-33), which was found to reduce the xanthine dehydrogenase activity by 50%, the aldehyde oxidase activity by 90%, and to virtually eliminate the pyridoxal oxidase activity. The third regulatory gene is known as aldox - 2 (map position 2-86), which reduces the aldehyde oxidase activity by 70%, but has no effect on the other two enzymes. The fourth regulatory gene in this system is the cinnamon gene (map position 1-0.0), which is a homozygous lethal mutation, and is characterized

by cinnamon larvae having very small amounts of these enzymes due to a maternal affect. This small activity disappears in the adult stage.

The mutation affecting the aldehyde oxidase and xanthine dehydrogenase activity in the hll6 strain was found to map to position 1-0.0. Therefore the emphasis in this discussion will be on comparing the hll6 enzyme deficiency mutation to the cinnamon mutation.

2. The hll6 Phenotype

Although the genes controlling the temperature sensitivity, and enzyme deficiency mapped to the same area of the X-chromosome, the first conclusion which can be made from these results is that the two phenotypes seen in the hll6 strain, the temperature sensitivity, and the enzyme deficiency, are the result of two separate mutations. The evidence for this comes from several different sources. First the recombination analysis resulted in one recombinant, the yellow 7 strain, which was temperature sensitive, but had wild type enzyme activity. This indicated that there had been a recombination event, separating the two mutations. The next piece of evidence comes from the cross of the sc8 strain to the hll6 strain, which resulted in the male progeny having wild type enzyme levels, due to the duplication of the

section of the X-chromosome onto the Y-chromosome. These males were still temperature sensitive, this indicating that the duplication covered the enzyme deficiency, but did not cover the temperature sensitivity. These two pieces of evidence taken together are a strong indication that there are two mutations responsible for the two phenotypes seen in the hll6 strain. The fact that the cal tech strain, and the w-hll6 recombinant strain were not temperature sensitive is further evidence for this. The map distance between the temperature sensitivity, and enzyme deficiency, was calculated to be 0.1 map units. Therefore it can be concluded that the mutant phenotypes of the hll6 strain are the result of two different mutations, one controlling the temperature sensitivity, and one controlling the enzyme deficiency. It is likely then that the temperature sensitive lethality is a separate mutation from the aldehyde oxidase regulatory system. It by itself may be interesting in its effect as a temperature sensitive mutation, but for the purposes of the study of the aldehyde oxidase regulatory system it can be excluded from consideration. Therefore, these two mutants will now be discussed separately.

## 2.a. The Enzyme Deficiency Mutation

The results of the characterization of the enzyme deficiency showed that the mutation resulted in a characteristic decrease in aldehyde oxidase activity. The comparison of the hll6 enzyme activity to the various genotypes, and the comparison of the recombinant strains showed that there was little variation in the enzyme activity of the enzyme deficiency mutation. Although there was variation seen in the recombinant strains, the enzyme deficiency mutation did not show a variation from near zero activity to that of near wild type enzyme activity. This result indicates that as far as the recombinants were concerned, the enzyme deficiency seemed to act as a single mutation. This mutant seemed to lower the activity by a certain percentage, throughout the development of the flies. The developmental comparison of the aldehyde oxidase between the hll6 strain and the wild type strain shows that the developmental profiles are the same, but in the hll6 strain, there is a much lower activity at all stages.

The enzyme deficiency mutation mapped to the same area as the cin mutation, which also has a deficiency in aldehyde oxidase. Therefore, it would seem possible that this mutant might be a naturally occuring allele of

cin. This was tested for by crossing the h116 strain with the cin-1 mutation which resulted in complementation between the two mutations. The resultant enzyme levels were slightly less that that of the yellow white wild type levels, but close to the aldehyde oxidase levels of the Canton S wild type strain. This suggests that the h116 is a different allele from that of the cin-1 allele. When the h116 strain was crossed to wild type strains, normal enzyme levels were seen. These pieces of information indicate that complementation with cinnamon did occur. The cinnamon mutation itself blocks the production of aldehyde oxidase, therefore in the adult no aldehyde oxidase is present. This was shown to be the case in this study also. The level of the h116 aldehyde oxidase was found to be approximately 12% of that of the yellow white strain. Since the heterozygotes have much greater enzyme activity than these mutations have separately, it is tempting to suggest that complete complementation has occured. However, this is difficult to establish due to the variation in the aldehyde oxidase activity in different wild type strains. As can be seen in the comparison of the aldehyde oxidase activity of different genotypes (Table  in results), the Canton S strain had approximately half the activity of the yellow white strain. This demonstrates the variation in activity

in different wild type strains. Since the activity of the wild type allele of the h116 strain is not known, it is not possible to say whether complete complementation has been achieved. The aldehyde oxidase activity of the h116 strain, before the enzyme deficiency mutation occured should be known before it is possible to say whether the complementation with the cinnamon has restored aldehyde oxidase activity of h116 to wild type levels. Another approach to this problem, is to examine the levels of activity in flies which are heterozygous for cinnamon, and a wild type allele. The cinnamon//yellow white heterozygotes, and the cinnamon//Canton S heterozygotes (Table 5 results section), showed no difference in enzyme activity from that of the wild type hemozygotes. Therefore, it would seen that cinnamon has no effect on reducing wild type alleles, and therefore the wild type alleles completely complemented the cinnamon mutation. As can be seen from the same table, there was also complete complementation between the wild type alleles, and the h116 enzyme deficiency. However, since the wild type levels of enzyme activity, of either the cinnamon, or the h116 strains are known, it is not possible to positively state that there is full complementation between the two mutations. Even so, since the activity of the h116// cinnamon heterozygote is as great as the Canton S strain,

it is safe to assume, that complementation is fully, or very near fully complete.

This complementation leads to the conclusion that the two mutations are not at the same genetic locus. This is consistent with the work of Bentley and Williamson (1979), who found different alleles of cin existed in Drosophila melanogaster. After inducing 16 alleles of cin with EMS (ethyl methanesulfonate) mutagenesis, Bentley and Williamson (1979) found that these fell into four complementation groups. Three of the induced alleles were viable when homozygous. When these alleles (termed cin-9, cin-11,and cin-15) were crossed with cin-1, near wild type enzyme levels were found. The cin 9//cin-1 heterozygote had 74% enzyme activity, for aldehyde oxidase. The cin 11// cin-1 and the cin-15//cin-1 heterozygotes both had enzyme levels greater than that of wild type.

On comparing the h116 allele to the 16 alleles induced by Bentley and Williamson, it would seem that the cin-9 allele was closest to the h116 allele. All of the induced alleles, with the exception of cin-9, cin-10, cin-11, and cin-15, were found to be inviable when homozygous. Of the four viable strains two, cin-10 and cin-11 had the cin eye colour. Therefore, the two cin alleles

which were most like the hll6-cin allele are the cin-9 and cin-l5 alleles. Of these two, the cin-9 showed complementation with cin-l more like the hll6-cin than the cin-l5 allele.

It would seem then that the hll6 allele falls into the complementation group C of Bentley and Williamson's (1979) complementation map of the cin region, and it would also seem that the hll6 allele is very similar to the cin-9 allele. It is possible that the hll6-cin allele is the same allele as the cin-9 allele due to the reasons discussed above. However, there are some important differences which suggest that these are two different alleles. In their 1979 paper, Bentley and Williamson state that the enzyme activities for the cin homozygotes is less than 15%. This is similar to the hll6 enzyme activity which has an activity of 12% of that of wild type activity. In their 1982 paper, Bentley and Williamson reported the developmental profiles of homozygotes and heterozygotes of the cin alleles 1, 9, 10 and 15, which shows the activity of the adult cin homozygotes to be actually less than 5% of that of wild type. The figures of the enzyme activities throughout development show that the enzyme activity for the homozygous cin strains starts off at around 5 to 10 units of activity, at the early third instar larvae stage,

and then decreases to virtually zero activity. The h116 homozygote showed an increase in activity at the third instar larvae stage, and then decreased slightly, but still within levels of detectability. Therefore, since both the developmental profiles of these two alleles are different, and they seem to have very different enzyme activities in the adult stage, it would seem the h116 allele is not the same as the cin-9 allele described by Bentley and Williamson (1982), and is in fact a new allele of cin. However, it is difficult to compare these two sets of data, because of the different techniques used to assay enzyme activity. Since Bentley and Williamson used partially purified enzyme, a large difference in results may be due to the different assay techniques. The final confirmation of this would be to actually compare the two alleles in a single experiment, using exactly the same assay techniques, to determine their exact degree of similarity. Crossing the two strains to determine if complementation could be obtained would not yield information as to whether these two alleles were the same ones or not. It would only provide information as to whether they fall into the same complementation group or not.

## 2.b. Temperature Sensitivity of the h116 Aldehyde Oxidase

Bentley and Williamson (1979) have reported information about the heat lability of the aldehyde oxidase produced by the cin alleles they generated. This was done by leaving the enzyme extract at 60°C for varying lengths of time to determine the rate at which it was denatured. This demonstrated the heat lability of the enzyme itself. From the results in this thesis, the aldehyde oxidase produced by the h116 allele was shown to be temperature sensitive. This, however, could be due to either the heat lability of the enzyme itself, or the heat lability of the processing mechanism of the cin allele. The fact that it was heat labile in vivo at the comparatively low temperature of 29°C (as compared to 60°C used to denature the enzyme) indicates that it is the processing mechanism which is temperature sensitive. If in fact this was shown to be the case, this could be a useful tool for the further study of the action of this allele. One possible method of studying its effect would be to determine the enzyme activity of the enzyme in heterozygotes at both 19°C and at 29°C. If the processing mechanism was heat labile, heterozygotes grown at 29°C should have half of the activity of heterozygotes grown at 19°C, due to only one of the processing alleles being

active. Therefore, the aldehyde oxidase should be processed half as fast, resulting in the fly having only half the activity. If the enzyme itself is heat labile, then complementation should occur, between the wild type allele and the hll6 allele, and there should be no difference in the enzyme activities, except any differences due to the increased heat. Studies of the heat lability at 29°C of the aldehyde oxidase produced by the hll6-cin strain, would also be useful in determining exactly what was affected by the heat. Another method of studying this allele and the effect of temperature would be to examine the electrophoretic banding patters of heterozygotes grown at 29°C and 19°C. This would allow one to be able to determine whether the primary sequence of the structural gene was altered by the cin alleles. If there was an effect of the cin alleles on the primary sequence, then a heterozygote between a wild type allele and the hll6 allele should result in an alteration of the banding pattern for aldehyde oxidase, at 29°C. This would be due to the one processing allele being active, and the other allele being inactive, or seriously altered.

Cipher and Courtright (1978) have reported that the product of the cinnamon allele was temperature sensitive, and essential to zygote survival. However, the strain used for their experiments was a spontaneously

generated cinnamon allele. The description given for the allele is very close to that of the h116 mutation. Therefore it is possible that it was the temperature sensitivity mutation responsible for the zygote survival, and not the cinnamon allele.

2.c. The Possible Action of the h116 Allele

Several of these experiments yield some information as to how the h116 mutation may work. The developmental profile of the h116 allele indicates that this mutation acts in a way that uniformly decreases the enzyme activity throughout development, rather than acting at a particular stage. The developmental profiles of the cin alleles induced by Bentley and Williamson show that these alleles have a completely different developmental profile than the wild type allele. These allele tend to completely stop enzyme activity, rather than reduce it.

Browder et al (1982), have determined CRM levels for the cin-1 and cin-9 alleles. Their results show that at the adult stage, the cin-9 allele has 32% of wild type cin CRM. Therefore the cin-9 allele does seem to have a low level of inactive cin CRM. This result would indicate that the cinnamon mutation has two effects.

The first effect is to reduce the amount of unprocessed enzyme produced by the structural gene. The second effect is to block the processing of this unprocessed enzyme into active enzyme. This experiment could be repeated to find if the h116 allele has a similar amount of CRM. By comparing the ratio of CRM to active aldehyde oxidase in the h116 strain, one could determine whether all the CRM is processed into active enzyme, or if only a small portion of the CRM is processed. This would yield information about the two functions of the cinnamon allele and how they worked in the cin-9 and h116 strains. The first possibility is that the h116 has the same amount of CRM as the cin-9 allele. If this were the case, then both functions (the production of unprocessed enzyme, and the production of active enzyme) would be partially operative in the h116 strain resulting in the low level of activity. If the level of CRM in the h116 strain was much higher, approaching wild type levels, this would indicate that the first function was restored in the h116 strain, and the second function was only partially restored. If the level of active enzyme was equal to the amount of CRM, this would indicate that the second function was completely restored, and the first function was only partially working. By comparing the aldehyde oxidase activities, and CRM levels in the cin-9 strain, and the h116 strain, it could also be determined whether or not these two were the same alleles or not.

The maroon-like mutation has been characterized in depth (see literature review). Its main function seems to be to donate a cofactor to the appoenzyme. However, the function of the cinnamon allele seems to be much more complex. The fact that four complementation groups exist is further evidence for this. If in fact the cinnamon allele does have two functions, this would indicate that it is a much more complex locus than the maroon like locus. The second function of cinnamon, the processing of inactive enzyme into active enzyme may be similar to that of the maroon-like locus. However, the first function of affecting the production of the unprocessed enzyme is much more difficult to deal with. One possibility, is that the cinnamon allele acts to process the primary RNA transcript of the structural gene, into a form where it can be then translated into the CRM. The second function could be to donate a cofactor to the newly translated CRM, which could then go on to be processed further by the other regulatory genes in the system.

3. The Temperature Sensitive Lethality Mutation

The results of the recombination analysis showed that the temperature sensitive lethality mutation mapped to 1-0.1. As previously stated, this was most likely not involved in the aldehyde oxidase regulatory system, and was a separate mutation. The results of the shift up and shift down experiment indicated that the period of sensitivity was throughout the larval periods of the flies, with survivorship increasing in the adult stages.

This mutant would be interesting in itself to study as a temperature sensitive mutation. The most obvious method of studying this mutation would be to determine what metabolic defect it caused. This could be accomplished by comparing the physiological changes, in the developing larvae and adults, at both 19°C and 29°C. It was observed that, as the h116 strain was grown at the higher temperature, small, black formations appeared after a few days. These formations were associated with the larval intestinal tract. As the larvae went through development, these formations increased in size until the third instar stage, when the larvae died. It would seem that these formations could be examined to determine their composition, which could yield important information about the exact defect caused by the temperature sensitive lethality.

# SUMMARY

A strain of Drosophila melanogaster (Mid America Stock Culture number h116) was found to be deficient in aldehyde oxidase and xanthine dehydrogenase activity (Hickey and Singh 1982). During the course of the present study, this strain was also found to be temperature sensitive with respect to its viability. Genetic analysis indicated that both mutant phenotypes (enzyme deficiency and temperature sensitive lethality) were controlled by X-linked genes. Recombination analysis showed that the two mutations were closely linked and in the yellow white region of the X-chromosome. Recombinants in this area were recovered, and it was found that the two mutant phenotypes were controlled by two different mutations. The enzyme deficiency was found to be inseparable from the yellow locus, while the temperature sensitive lethality was found to map to position 1-0.1. Crossing the h116 strain to a scute-8 strain which had a duplication of the X-chromosome (covering map position 0.0) translocated onto the Y-chromosome, yielded male progeny which were temperature sensitive, but had wild type levels of aldehyde oxidase activity, thus conforming that there were two mutations responsible for the two h116 phenotypes.

The enxyme deficiency mutation and the temperature sensitive lethal mutation were characterized separately. The aldehyde oxidase activity was first compared to enzyme activities of various wild type strains. It was found that the hll6 strain had approximately 12% of the activity of that of wild type. The developmental profile of the enzyme activity revealed that the hll6 aldehyde oxidase activity was not detectable until the third instar larvae stage, while the wild type levels were detectable at the second instar larvae stage. The hll6 aldehyde oxidase activity decreased toward the adult stages, while the wild type activity increased in the third instar larvae stage, and then decreased slightly toward the adult stage. The aldehyde oxidase activity of the hll6 strain was also found to be temperature sensitive. Flies which were not temperature sensitive lethals were raised at 29°C, and assayed for their enzyme activity. It was found that the hll6 aldehyde oxidase activity dropped below detectable levels in flies raised at the higher temperatures, while in the wild type the enzyme levels remained unchanged. Various heterozygotes between the hll6 and other wild type strains were made and assayed for aldehyde oxidase activity. It was found that the aldehyde oxidase was complemented by the wild type alleles, but complementation was seen for the hll6 enzyme deficiency

mutation and the cinnamon mutation, indicating that the h116 enzyme deficiency mutation was a separate mutant from the cinnamon mutation.

The temperature sensitivity mutation was characterized for its action in the developmental period of the flies' life cycle. This was accomplished by the use of "Shift Up" and "Shift Down" experiments, in which the flies were shifted either up to, or down from the lethal temperature at various stages in their life cycle. It was found that the lethality acted throughout the egg and larval periods, until the later adult stages. After the h116 flies reached the adult stages, the temperature sensitivity decreased.

# CONCLUSION

The results clearly demonstrate that the h116 strain has two distinct mutations, one which is responsible for the temperature sensitivity, and one which results in the enzyme deficiency. These were both mapped to the X-chromosome, near or at position 0.0. The fact that there are two mutations is demonstrated by the y-7 strain, generated by the recombination experiment. This strain was found to be temperature sensitive lethal, but had wild type enzyme levels. The crosses with the sc8 strains, producing F1 males which were heterozygous for the enzyme deficiency, but hemizygous for the temperature sensitivity, also demonstrates that there are two separate mutations. The fact that the cal tech h116 strain, and the w-h116 strain were not temperature sensitive is further evidence for this. The map distance between the two mutants is calculated to be 0.1 map units. Therefore, the temperature sensitive lethal mutation was concluded to be a separate mutation from that of the enzyme deficiency.

The enzyme deficiency was determined to be a new allele of cin, on the basis of its complementation with the cin 1 allele, and on comparing it with other previously described mutations. It was postulated that it might act to uniformly lower enzyme activity throughout the flies' life cycle.

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# APPENDICES

## SHIFT UP EXPERIMENT

| Rep | 3rd Instar Larvae | | Pupae(Early) | | | Pupae(Late) | | | One Week Old Adults yw | | | h116 | | Controls | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No. | yw | h116 | No. | yw | h116 | No. | yw | h116 | No. | Shifted | Survivors | Shifted | Survivors | No. | yw | h116 |
| 1 | 19 | 0 | 1 | 33* | 0 | 1 | 0 | 0 | 1 | 5 | 5 | 2 | 1 | 1 | 7 | 5 |
| 2 | 15 | 0 | 2 | 25 | 0 | 2 | - | - | 2 | 7 | 7 | 3 | 3 | 2 | 16 | 6 |
| 3 | 12 | 0 | 3 | 21 | 0 | 3 | 25 | 0 | 3 | 11 | 11 | 4 | 3 | 3 | 14 | 3 |
| 4 | 24 | 0 | 4 | 17 | 0 | 4 | 18 | 0 | 4 | 25 | 25 | 5 | 2 | 4 | 3 | 1 |
| 5 | 19 | 0 | 5 | 13 | 0 | 5 | 13 | 0 | 5 | 11 | 11 | 4 | 4 | 5 | 11 | 4 |
| 6 | 18 | 0 | 6 | 4 | 0 | 6 | 20 | 0 | 6 | 21 | 21 | 8 | 8 | 6 | 8 | 3 |
| 7 | 12 | 0 | 7 | 17 | 0 | 7 | 14 | 0 | 7 | 8 | 8 | 6 | 8 | | | |
| 8 | 17 | 0 | 8 | 16 | 0 | 8 | - | - | 8 | 9 | 9 | 3 | 2 | | | |
| 9 | 21 | 0 | 9 | 14 | 0 | 9 | 0 | 0 | 9 | 16 | 16 | 7 | 4 | | | |
| 10 | 13 | 0 | 10 | 16 | 0 | 10 | 10 | 0 | 10 | 17 | 17 | 8 | 3 | | | |
| | 170 | 0 | | 139 | 0 | | 100 | 0 | | 130 | 130 | 50 | 36 | | 54 | 22 |

In all experiments, 25 eggs or pupae were transferred to each vial.

In late pupae and adult experiments, survivorship is measured as number of adults alive after one week.

* - Not used in totals

## SHIFT DOWN EXPERIMENT

| Immediately Shifted (12 hrs.) | | | 1d | | | 2d | | | 4d | | | 8d | | | Controls | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Rep No. | yw | h116 | Rep No. | yw | h116 | Rep No. | yw | h116 | Rep No. | yw | h116 | Rep No. | yw | h116 | Rep No. | yw | h116 |
| 1 | 8 | 0 | 1 | 9 | 0 | 1 | 20 | 0 | 1 | 17 | 0 | 1 | 8 | 0 | 1 | 7 | 0 |
| 2 | 13 | 0 | 2 | 12 | 0 | 2 | 17 | 0 | 2 | 11 | 0 | 2 | 13 | 0 | 2 | 6 | 0 |
| 3 | 10 | 0 | 3 | 10 | 0 | 3 | 15 | 0 | 3 | 9 | 0 | 3 | 10 | 0 | 3 | 8 | 0 |
| 4 | 14 | 0 | 4 | 14 | 0 | 4 | 16 | 0 | 4 | 14 | 0 | 4 | 16 | 0 | 4 | 16 | 0 |
| 5 | 10 | 0 | 5 | 16 | 0 | 5 | 20 | 0 | 5 | 20 | 0 | 5 | 14 | 0 | 5 | 15 | 0 |
| | 55 | 0 | | 61 | 0 | | 80 | 0 | | 71 | 0 | | 61 | 0 | | 52 | 0 |

In all experiments 25 eggs or individuals were transferred per vial.